Department of Forensic Science

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FORENSIC BIOLOGY PROCEDURES MANUAL

VIRGINIA DEPARTMENT OF FORENSIC SCIENCE

QUALITY ASSURANCE PROGRAM

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1 QUALITY ASSURANCE PROGRAM

1.1 Goals and Objectives

1.1.1 Goals

- To support criminal justice agencies in the Commonwealth with DNA analysis of selected biological materials associated with criminal and missing persons investigations using polymerase chain reaction (PCR) based testing.
- To maintain a DNA Data Bank of convicted offenders and arrestees.
- To ensure the quality, integrity, and accuracy of the analytical data through the implementation of a detailed quality control program.

1.1.2 Objectives

- Establish and monitor quality requirements for reagents, supplies, equipment, and analytical procedures.
- Ensure that DNA analytical procedures are operating within the established performance criteria and that the quality and validity of the analytical data is maintained.
- Ensure that problems are noted and that the appropriate corrective action is taken and documented in a Memorandum of Record or case file documentation for the affected case file(s) and, if necessary, for the Quality Control records.

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1.2 Organization and Management Structure

The organization and management of the Virginia Department of Forensic Science is addressed in the Department Quality Manual.

The management structure for the Department is set forth in the Department Organizational Chart.

The contingency plan for a vacated Technical Leader position is detailed in the Department Quality Manual.

If at any time the number of qualified Forensic Biology analysts statewide falls below two full-time employees who are qualified analysts, casework in the affected discipline or sub-discipline or Data Bank sample processing, as applicable, will be halted. The Biology Program Manager (Technical Leader) will then determine the appropriate next steps in reinstating the discipline, sub-discipline, or Data Bank sample processing.

1.3 Personnel Qualifications and Training

The procedure for the qualification and training of personnel is addressed in the Department Quality Manual.

The policy that addresses attending meetings and seminars is addressed in the Department Quality Manual, Continuing Education and Training, and associated documentation is considered part of the overall training record for document retention purposes.

Each laboratory will maintain the training record for each assigned person.

All analyst(s), the technical leader and casework CODIS administrator(s) must receive 8 hours of continuing education on a yearly basis. The technical leader shall approve the continuing education program which will be documented in Qualtrax.

A review of scientific literature will be conducted through the use of a reading list. The technical leader will approve the articles on the list. Documentation of the completion of the required reading shall be maintained by the section supervisor or their designee(s).

1.4 Facilities

The Facilities of the Department are addressed in the Department Quality Manual.

1.5 Evidence Control

The procedure for the assignment of laboratory numbers to submission of physical evidence, and item [sub-item] numbers to evidence contained in the submission of physical evidence is addressed in the Department Quality Manual.

The procedure for the handling of physical evidence is addressed in the Department Quality Manual. Work product is defined as the material that is generated by the laboratory as a function of analysis that is not subject to a chain of custody and includes amplified product and amplification tubes or plates.

1.5.1 All DNA extracts and cuttings to be returned to the submitting agency (refer to FB PM Extraction of DNA) for possible future testing are securely maintained in the Forensic Biology laboratory in which they were created during the normal workflow process until they are placed into the applicable associated container for return.

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1.5.1.1 Exceptions are specifically addressed in 1.5.2.3.

1.5.2 A chain of custody will be maintained for DNA extracts and/or cuttings that will be returned to the submitting agency for possible future testing.

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1.5.2.1 Creation of a sub-item in the LIMS is not typically required.

1.5.2.2 The chain of custody will be documented in the case notes and will minimally include:

- Date of creation and initials of the creator
- Date placed into the applicable associated container for return to the submitting agency and initials of person performing this task

1.5.2.2.1 Movement within the Forensic Biology laboratory during the normal workflow process (e.g., storage after robotic extraction and normalization, reamplification of an extract, drying down of extracts, storage after drying, etc.) occurs in a controlled access environment and need not be documented.

1.5.2.2.2 The creation of a cutting can be captured in the case file notes (i.e., the date and initials for the notes page will suffice) and is defined as:

- the placement of a cutting taken directly from an item of evidence into a tube for possible extraction

OR

- or the placement of a cutting from a swabbing taken directly from an item of evidence into a tube for possible extraction

1.5.2.2.3 The creation of an extract is the placement of the first extraction reagent into the tube in which the extraction is to take place.

1.5.2.2.3.1 For a batch composed of samples from more than one examiner, handwritten initials of each person performing each type of extraction next to the date of extraction already documented on the batch paperwork will suffice.

1.5.2.2.3.2 For a batch composed of samples from a single examiner, the handwritten initials at the top of the page required for all casework documentation will suffice.

1.5.2.2.3.3 Sperm and non-sperm fractions are considered to have been created on the date on which the extraction begins, even if the tube is incubated overnight at 37ºC prior to transfer of the non-sperm portion into a separate tube.

1.5.2.3 Internal transfers of DNA extracts and/or cuttings outside of their applicable associated container to a member of another section, to a different examiner of the same section for the purposes of performing a type of testing in which the originating examiner is not qualified, or to another VA DFS laboratory via lockbox will be documented in the LIMS.

1.5.2.3.1 This type of transfer will require that the affected extracts and/or cuttings be sub-itemized in the LIMS and the transfers tracked electronically.

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EXAMPLES: A DNA extract is created by a mitochondrial DNA examiner who is not qualified to perform Y-STR analysis. The extract is later referred for Y-STR analysis. This extract must be sub-itemized in the LIMS and the associated transfers to and from the nuclear DNA section/examiner and the mitochondrial DNA examiner tracked electronically.

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A DNA extract is created by a nuclear DNA examiner in the Central Laboratory who is not qualified to perform Y-STR analysis. The extract is later referred for Y-STR analysis and transferred to a different examiner in the Central Laboratory for the Y-STR analysis. This extract must be sub-itemized in the LIMS and the associated transfers to and from the nuclear DNA section/examiners tracked electronically.

A DNA extract is created by a nuclear DNA examiner in the Western Laboratory. The extract is later referred to an examiner in the Central Laboratory for Y-STR analysis. This extract must be sub-itemized in the LIMS and the associated transfers to and from the Western DNA section/examiner and the Central DNA section/examiner tracked electronically.

A DNA extract is created by a nuclear DNA examiner in the Central Laboratory. This extract is amplified as a part of the normal workflow of a batch of samples from multiple examiners. The amplification positive control fails and requires a reamplification of the entire batch of samples. One examiner with samples in the batch offers to reamplifiy the entire batch manually. This is defined as movement during the normal workflow process that will be documented through the use of the manual amplification set up worksheet. Therefore, it need not be documented in the notes or in the LIMS.

1.5.2.4 Once placed into the applicable associated container for return to the submitting agency, the chain of custody of the parent container encompasses the chain of custody of the extract(s) and/or cutting(s). Any transfers after this point do not require sub-itemization of an affected extract and/or cutting that was not previously sub-itemized.

1.5.3 A chain of custody will be maintained for all microscope slides created in the laboratory that will be returned to the submitting agency for possible future testing (e.g., 3µL spots of sperm fractions that are not discarded due to no spermatozoa having been observed, positive slides created from stains on bedding, etc.).

1.5.3.1 Creation of a sub-item in the LIMS is not required.

1.5.3.2 The chain of custody will be documented in the case notes and will minimally include:

- Date of creation and initials of the creator – the date and initials for the notes page will suffice.
- Date placed into the applicable associated container for return to the submitting agency and initials of person performing this task – if conducted and documented on the same day and on the same original notes page as the creation, the date and initials for the notes page will suffice.

1.5.3.3 Once placed into the applicable associated container for return to the submitting agency, the chain of custody of the parent container encompasses the chain of custody of these laboratory created microscope slide(s).

1.6 Validation

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The validation of new software, software changes, and new DNA technologies/methodologies by the Forensic Biology Section is addressed in this manual. In addition, a summary of each validation study is maintained with the data. The summary and data are maintained by the Biology Program Manager as directed by the Department Quality Manual.

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1.7 Analytical Procedures

The analytical procedures used by the Section are set forth in the Forensic Biology Procedures Manuals and the Mitochondrial DNA Section Procedures Manual. Establishment of these procedures is guided by the Department Quality Manual.

1.7.1 Estimation of the uncertainty of measurement: no critical measurements are taken during the analytical process in the Section; therefore, no estimation of uncertainty of measurement is necessary.

1.8 Calibration and Maintenance

Procedures for the calibration and maintenance of laboratory equipment are set forth in this manual. Establishment of these procedures is guided by the Department Quality Manual.

1.9 Proficiency Testing

The proficiency testing program for the Department is referenced in the Department Quality Manual.

1.10 Corrective Action

The procedure for corrective action is set forth in the Department Quality Manual.

1.11 Reports

The procedure for generating a Certificate of Analysis is addressed in the Department Quality Manual.

The procedure for the preparation, storage and disposition of case file records is addressed in the Department Quality Manual.

The procedure for the expungement of records is addressed in the Department Quality Manual.

1.12 Review

The Department procedure for the administrative and technical review of case files is addressed in the Department Quality Manual. To aid the Section examiner during the review of the documentation found in the case file, the "Guide for Review of Autosomal DNA Data" found in Appendix J and the "Guide for Review of Y-STR DNA Data" found in Appendix K may each be used as a guide and are not intended to supercede or replace the official technical review form addressed in the Department Quality Manual.

Although the technical and administrative reviews are conducted as a combined review in accordance with the Department Quality Manual, the completion of these reviews will be documented separately with the reviewer's handwritten initials or signature and date of each review on a Department technical review form (TRF) to be maintained in each case file. The same TRF may be used to document a nonconformity, its resolution, and the completion of the technical and administrative reviews, as applicable. Only TRFs used to document a nonconformity must be forwarded to the examiner's supervisor and subsequently the Laboratory Director for review, as detailed in the Department Quality Manual.

Forensic Biology casework combined technical and administrative review will include:

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- a review of all case notes, all worksheets, and the electronic data (or printouts of such data) supporting the results and/or conclusions.
- a review of all analytical controls, internal size standards, allelic ladders used for sizing, and analysis methods/parameters to verify that the expected results are obtained.
- a review of the raw data for reagent blanks and negative controls to ensure amplification product was indeed loaded into the plate for the Genetic Analyzer.
    - A review of the raw data associated with evidence samples or known reference samples for which no typing results are obtained may be indicated if the quantitation value for the sample indicated a profile should have been obtained.
- a review of all DNA types to verify that they are supported by the raw or analyzed data (electropherograms or images).
- a review of all data to verify conclusions (i.e., inclusions, exclusions, inconclusive) are in compliance with laboratory guidelines.
- a review of statistical analyses, if applicable.
- a review of the case file and final report's content to verify compliance with QAS Standard 11.2 and that the results and/or conclusions are supported by the data.
- verification that all profiles entered into CODIS are eligible, have the correct DNA types, and are entered into the correct specimen category.
- a review of the case file and final report for clerical accuracy.
- a review of the chain of custody and disposition of the evidence.
    - The chain of custody review includes the written chain of custody on the RFLE and the electronic chain information maintained in FA.

On occasion, there can be initial disparities between the conclusions drawn by qualified examiners in the technical review process. The first step towards achieving a unified scientific conclusion should be a discussion between the examiner and technical reviewer. In this discussion, each party should provide details supporting the basis of his/her conclusions. If consensus cannot be achieved, a supervisor should be consulted. The Biology Program Manager (Technical Leader) or Assistant Technical Leader can be contacted for further technical discussions between the examiner and technical reviewer/supervisor or examiners in other laboratories, if necessary. An inconclusive result should be reported in the rare instance where a scientific consensus cannot be reached.

Data Bank combined technical and administrative review will include:

- a review of all notes, all worksheets, and the electronic data (or printouts of such data) supporting the results and/or conclusions.
- A review of all analytical controls, internal size standards, allelic ladders used for sizing, and analysis methods/parameters to verify that the expected results are obtained, except if using an NIDS approved and internally validated Expert System.

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- a review of all DNA types to verify that they are supported by the raw or analyzed data (electropherograms or images), except if using an NDIS approved and internally validated Expert System.

An additional documented annual review of Forensic Biology case files independent of the required combined technical and administrative review process and of any external audit conducted in accordance with the FBI Quality Assurance Standards shall be conducted. The scope and plan for this annual review will be determined prior to its start each applicable year and will include a representative sample of cases worked, as determined and approved by the Biology Program Manager (Technical Leader).

An additional documented annual review of Data Bank sample processing records independent of any external audit conducted in accordance with the FBI Quality Assurance Standards shall be conducted. The scope and plan for this annual review will be determined prior to its start each applicable year and will be representative of the samples tested, as determined and approved by the Biology Program Manager (Technical Leader).

The procedure for the monitoring of the testimony of casework examiners is addressed in the Department Quality Manual.

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1.13 Safety

The policies and procedures for safety in the laboratory are addressed in the Department Safety Manual.

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1.14 Audits

The procedure for audits of the Department is addressed in the Department Quality Manual.

A FBI Quality Assurance Audit of the Section is conducted annually, as per the Department Quality Manual.

1.15 Outsourcing

Outsourcing is addressed in the Department Quality Manual.

Technical specifications of the outsourcing agreement are approved by the technical leader prior to the outsourcing agreement being awarded.

A technical review of the vendor's data is conducted prior to uploading sample profiles into SDIS. The following elements are included in the technical review:

- DNA types/haplotypes to ensure they are supported by the analytical data
- Associated controls, ILS and ladder, as applicable to the technology employed
- A review of the final report if provided by the vendor
- Verification of the DNA types/haplotypes, eligibility and correct specimen categories for entry to CODIS

A documented initial on-site visit to assess the vendor laboratory's ability to perform analysis on outsourced casework, prior to the vendor laboratory's beginning of casework analysis will be conducted by the Biology Program Manager/Technical Leader or a designated employee, unless the Technical Leader evaluates and approves an on-site visit coordinated by a designated FBI employee.

If the outsourcing agreement extends beyond one year, an annual on-site visit shall occur, unless the Technical Leader reviews and approves an on-site visit conducted by another NDIS participating laboratory using the same technology, platform, and typing test kit for the generation of the DNA data, or coordinated by a designated FBI employee.

1.16 Document Retention

The document retention policy of the Department is addressed in the Department Quality Manual.

1.17 Case File Documentation and Storage of Electronic Data

Technical records that are generated electronically and printed are considered complete after the document is printed and the print session is closed. Changes to these records after this time must be tracked and made in accordance with the Department Quality Manual.

Technical records that are generated electronically and saved for printing upon completion are considered complete when the last analytical procedure documented on that record (e.g., worksheet) has been completed. Changes to these records after this time must be tracked and made in accordance with the Department Quality Manual.

EXAMPLE: Within the Excel workbooks used for DNA analysis, each individual worksheet will be considered complete once the last procedure documented on that worksheet has been completed.

Examination documentation that is saved electronically and not printed is considered complete when saved to a disk (or other suitable media) which is then initialed, dated and uniquely identified by the examiner prior to the technical review. Changes to this documentation after it is complete will need to be regenerated electronically and saved to the original disk or to a new disk which is then initialed, dated and uniquely identified by the examiner. The original and/or subsequent disks are saved as part of the case record.

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STR Testing:
Electronic sample files (.hid) and project files (.ser) generated using the 3500xL Genetic Analyzer and analyzed using the GeneMapper® ID-X software will be saved onto a storage medium (disk or other suitable media) and stored in each case file. The sample files to be saved include any/all sample files generated for the case, whether they are the data used for reporting or not (reinjections, etc.) and all associated reagent blank, negative control, and positive control sample files as well as any ladder(s) used in the sizing of samples in projects. The project file(s) (.ser) saved will be those that contain reported data. If, for example, a new project is created due to no passing ladders in the first project, the original project need not be saved.

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mtDNA Sequencing:
Electronic sample data files (.ab1) generated using the 3500xL Genetic Analyzer will be saved onto a storage medium (disk or other suitable media) and stored in each case file. The sample files to be saved include any/all sample files generated for the case, whether they are the data used for reporting or not (reinjections, etc.), all associated reagent blank, negative control, and positive control sample data files, and the Sequencher project files (.spf) generated by the reporting examiner/author of the Certificate of Analysis.

1.18 Redaction of Data Bank Information From Case File Documentation Released to Outside Parties

Identifiers and genotypes of eliminated candidate matches will be redacted from any case file documentation released to outside parties.

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2 EQUIPMENT, REAGENTS AND SUPPLIES

The purpose of the procedures in this section is to ensure that the parameters of the testing process are routinely monitored in the manner necessary to maintain the success and reliability of the testing procedures. Upon the completion of the quality control testing of critical reagents the supervisor or designee will review, initial and date the quality control documentation prior to the critical reagent being used to conduct casework or Data Bank analyses.

Controls utilized during each phase of the testing procedure are designed to signal potential problems in the analysis. If acceptable results are obtained for these controls, it is reasonable to assume that the results for other samples analyzed using the same equipment, reagents, and supplies will be accurate and acceptable. If the controls indicate a problem with the analysis, it may be possible to determine the source of the problem and correct for it. Depending on the nature of the problem, re-analysis of the samples may be required.

Where the samples are irreplaceable and/or limited in amount (as in most casework), it is highly desirable to minimize the need for repeat analysis due to failure of equipment, materials or reagents. To that end, quality control (QC) procedures should focus as much as possible on preventing problems before they occur rather than on dealing with them after they happen.

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2.1 Instruments and Equipment

2.1.1 Inventory

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The section will maintain an equipment inventory.

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2.1.2 Operating Manuals

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Warranty information and operating manuals will be filed in the laboratory or readily available online or through the vendor(s) and will be available to all operators of instruments and equipment.

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2.1.3 Calibration/Maintenance/Repair Records

Anytime an instrument or piece of equipment requires calibration, service, or maintenance, that fact will be documented on an "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form. Additionally, instruments/equipment on routine service contracts will have routine service calls documented. These forms will be maintained in a ring binder or properly labeled file.

2.1.4 Calibration and Maintenance Schedules

Routine maintenance and/or calibration will be performed on each instrument/piece of equipment listed, based upon the following schedule.

Critical equipment is defined as:

- NIST traceable thermometer
- Balance
- Heat blocks
- Incubators, unless used only to dry glassware
- Thermal cycler temperature verification system
- Thermal cycler
- qPCR system
- Genetic Analyzers
- Pipettes
- BIOMEK®

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NOTE: If an instrument/equipment is out of calibration with the manufacturers or the Section's specifications, the instrument/equipment will immediately be taken off line for repair and the maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form". If an instrument/equipment is out of calibration and cannot be repaired it will be taken off line and the attempted repair will be documented on the "Equipment Calibration/Maintenance/Repair Form". Subsequently, the instrument/equipment will be removed from the Section's inventory and will be documented.

- **ANALYTICAL BALANCES:**

  Annually - Serviced and calibrated by outside vendor. Record the service call on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

  If the balance is taken off line for repair/maintenance, prior to being put back in service, the annual calibration will be conducted or the calibration check will be conducted as a performance check.

  Calibration check - Because there are many different analytical balances used throughout the Section the weights used to verify the calibration of the analytical balance should include at least 3 data points covering the range for which the analytical balance is used.

  PASS/FAIL RANGES FOR EACH DATA POINT (WEIGHT)

0.1 g	± 0.005 g
1.0 g	± 0.01 g
10 g	± 0.1 g
50 g	± 0.1 g
100 g	± 0.5 g
400 g	± 1.0 g
500 g	± 1.0 g
1.0 kg	± 1.0 g

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  If the analytical balance is outside of the acceptable range perform the calibration check a second time prior to making a service call.

- **AUTOCLAVE:**

  6 Months - Verify and record operating temperature and pressure using a biological indicator. (Refer to Appendix A for the procedure.)

  Each Use - Check the temperature and pressure gauges to ensure the autoclave is working properly. Monitor autoclave tape indicator.

  As Needed - Clean. Have authorized vendor service.

  If the autoclave is taken off line for repair/maintenance, prior to being put back in service, the biological indicator test will be conducted as a performance check.

- **BIOMEK® NX^{P} WORKSTATION:**

  Annually - Have authorized vendor evaluate and perform necessary maintenance and repair. Record the service call on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

  Annually – The syringes and or pipettes on the instrument will be calibrated by an authorized vendor. Record the service call on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

Quarterly - Calibration of the Biomek® NX^{P} Automation Workstation – Refer to Appendix B for this procedure.

If the BIOMEK ® NX^{P} is taken off line for repair/maintenance, prior to being put back in service, the home all axes and framing the deck functions will be conducted as well as extracting samples and blanks as a performance check. The number of samples and blanks may vary depending on the repair/maintenance performed. Most situations will be covered by extracting 2 samples and 2 blanks and taking these samples all the way through the DNA typing process. If a pipette probe is out of calibration and repaired, a sample and a blank should be processed in this affected row. This will dictate if additional samples and blanks are processed. For example, if 4 pipette probes are out of calibration, then 4 samples and 4 blanks will be required for the performance check.

- **CENTRIFUGES:**

Annually - Using a photoelectric tachometer check and record the speed of the rotor. (Refer to Appendix C for the procedure.)

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If a centrifuge is only used to pulse spin samples it is not necessary to quality control check the centrifuge rotor speed using a tachometer.

Each use or as needed - Wipe out the inside of the centrifuge with 70% ethanol or isopropanol.

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As Needed - Clean and replace brushes. Have authorized vendor service and calibrate. Document the authorized vendor's service or the in-house maintenance on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

If a centrifuge is taken off line for repair/maintenance, prior to being put back in service, the annual tachometer check will be conducted as a performance check. This performance check is not required for centrifuges that are used for pulse spins only.

- **DNA CONCENTRATOR/EVAPORATOR:**

Each use or as needed - Wipe out the inside of the DNA concentrator/evaporator with 70% ethanol or isopropanol.

As Needed - Clean solvent collection trap.

- **FREEZERS (- 20°C):**

Weekly (and prior to putting into service) - Check and record temperatures each Monday. If the temperature checks cannot be performed on Monday, they will be made on the first regularly scheduled work day. If the temperature is greater than -10°C the thermostat will be adjusted to lower the temperature. Afterward the temperature will be monitored to ensure that the temperature does not increase. If the temperature continues to rise above -10°C the freezer will be removed from service until it can be repaired. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form found in Document 210-F600 or comparable log form.

- **HEAT BLOCKS:**

Weekly (and prior to putting into service) - Check and record temperature. If the temperature on the thermometer while in the hole of the block designed to hold the thermometer is greater than ± 2.0°C from the specified temperature (e.g., 37°C, 56°C, or 95°C) the thermostat will be adjusted to obtain the correct temperature. The heat block temperature will be re-read and documented. If the correct temperature cannot be achieved the heat block will be removed from service until it can be repaired. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

Each Use - Check temperature.

- **HOODS:**

  - **BIOLOGICAL SAFETY CABINETS (EVIDENCE HANDLING AND PCR SETUP) and FUME HOODS**

    Annually - Have authorized vendor evaluate and perform necessary maintenance and repair. Record the service call on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

    Each Use - Wipe down the inside of the hood with 10% bleach (or a solution that will degrade DNA) followed by cleaning with isopropyl alcohol and/or ethanol before each use. If the hood is equipped with an alarm and the alarm sounds when the hood is in use, and will not reset, the hood will be taken off line until it can be repaired. An authorized vendor will be contacted to repair the hood. The maintenance/repair will be documented.

  - **BENCH TOP AIRCLEAN WORKSTATIONS (EVIDENCE HANDLING, PCR SETUP and EXTRACTION) and DRYING CABINETS (EVIDENCE HANDLING)**

    Annually - Have authorized vendor evaluate and perform necessary maintenance and repair. Record the service call on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

    As Needed - Change pre-filter and HEPA filter.

    Each Use - Wipe down inside of the hood with 10% bleach. Do not use isopropanol or ethanol in the workstation.

- **INCUBATORS (OVENS):**

  If the incubator is used only to dry glassware it is not necessary to verify the temperature as specified below.

  Weekly - Check and record temperatures. If the temperature is greater than ± 2.0°C from the specified temperature (e.g., 37°C, 56°C, or 95°C) the thermostat will be adjusted to obtain the correct temperature. The incubator temperature will be re-read and documented. If the correct temperature cannot be achieved the incubator will be removed from service until it can be repaired. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

  Each Use - Check temperature.

- **NIST TRACEABLE THERMOMETER:**

  Purchase a new National Institute of Standards and Technology (NIST) traceable thermometer when the unit needs to be re-calibrated. Maintain documentation of the calibration of the NIST traceable thermometer. Prior to use or yearly check documentation to ensure thermometer is within calibration.

- **pH METER:**

  Each Day of Use or Per Manufacturer's Instruction, whichever comes last - Check and record the calibration at two set points.

  As Needed - Check solution in probe and replace. Document the maintenance on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

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If a pH meter is taken off line for repair/maintenance, prior to being put back in service, the each day of use check will be conducted as a performance check.

- **PHOTOELECTRIC TACHOMETER:**

  Follow manufacturer's requirements for recalibration – If required have an authorized vendor evaluate and recalibrate or recertify the tachometer. Document the maintenance on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

- **PIPETTES:**

  Annually - Have an authorized vendor evaluate and perform necessary maintenance and repair. Record this service on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form and retain the calibration certificate generated by the vendor. This serves to demonstrate that the pipette has passed QC prior to being put back into service.

  As Needed - Clean inside/outside with isopropyl alcohol and check the seal. (Refer to Appendix D for the procedure.) Once the pipette has been reassembled conduct a quick calibration check using the graduated Rainin Pipette Tips discussed in Appendix D. If a pipette appears to be out of calibration, it will be sent to an authorized vendor for repair. Record this service on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form and retain the calibration certificate generated by the vendor.

  As Needed - If a pipette appears to be out of calibration between normally scheduled performance/calibration checks, the pipette will be sent to an authorized vendor for repair. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form and retain the calibration certificate generated by the vendor which will serve as the pipette's performance check.

- **MULTI-CHANNEL PIPETTES:**

  Annually (if used for precision measurements) - Have an authorized vendor evaluate and perform necessary maintenance and repair. Record this service on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form and retain the calibration certificate generated by the vendor.

  If a multi-channel pipette appears to be out of calibration between normally scheduled performance/calibration checks, the pipette will be sent to an authorized vendor for repair. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form and retain the calibration certificate generated by the vendor which will serve as the pipette's performance check.

- **REFRIGERATORS (4°C):**

  Weekly - Check and record temperatures each Monday. If the temperature checks cannot be performed on Monday, they will be made on the first regularly scheduled work day. The temperature should fall between 1-6°C. If not, the thermostat will be adjusted accordingly to bring the temperature into this range. Afterward the temperature will be monitored to ensure that the temperature does not fall outside of this range. If the temperature continues to fall outside of this range the refrigerator will be removed from service until it can be repaired. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

- **STRATAGENE® MX3005P™ qPCR SYSTEM:**

  Semi-Annually – Instrument qualification Test – Refer to Appendix E for this procedure.

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NOTE: The filter/gain settings need to be changed back to the instrument default prior to conducting the test.

Semi-Annually – While the block is at room temperature, the wells of the thermal block should be cleaned with cotton swabs and ethanol or isopropyl alcohol to eliminate any block contamination that can cause signal noise. The hot top that lifts up may be cleaned with Type I water and a lint-free lab wipe or cotton swab (the top, underside and openings) – being sure not to drip any liquid inside the instrument while cleaning.

If the qPCR system is taken off line for repair/maintenance, prior to being put back in service, the instrument qualification test run will be conducted as a performance check.

- **THERMAL CYCLER VERIFICATION UNIT:**

Annually - Send the thermal cycler verification unit to an authorized vendor for calibration. Record the calibration/maintenance check on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form, and retain the calibration certificate generated by the vendor.

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- **THERMAL CYCLER:**

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9700 Thermal Cycler: Quarterly - Perform and record the results of the temperature calibration, temperature non-uniformity and the system performance diagnostic tests. If any of the tests fail, contact an authorized vendor. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form found in Document 210-F600 or comparable log form. Record the results of the verification test on the appropriate worksheet provided in Document 210-F601. Refer to Appendix G for the procedure.

If a thermal cycler is taken off line for repair/maintenance, prior to being put back in service, the quarterly calibration tests will be conducted as a performance check.

- **THERMOMETERS:**

Annually - Check calibration of each thermometer (excluding those with current certifications from NIST and thermometers used to verify refrigerator and freezer temperatures) in use against a NIST certified or NIST traceable thermometer. Refer to Appendix F for procedure. If the temperature is greater than ± 1.0°C from the temperature of intended use (e.g., 37°C, 56°C, or 95°C) the thermometer will not be used to monitor temperatures at that range. If the thermometer is greater than ± 1.0°C for more than one temperature range the thermometer will be removed from service and documented.

Check the calibration of all new thermometers (excluding NIST certified thermometers and thermometers that are to be used to verify refrigerator and freezer temperatures) against a NIST certified or NIST traceable thermometer prior to putting into service.

As Needed - Clean with mild detergent.

NIST certified thermometers will be considered as calibrated until their expiration date.

- **TYPE 1 WATER SYSTEM:**

As Needed - Change filters/cartridges, sanitize, and/or have an authorized vendor clean and check unit. Record the replacement/maintenance check on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

- **UV CROSSLINKER:**

  Quarterly – Determine the amount of time required to irradiate laboratory tube racks, disposables and ultra-pure water in order to achieve a sterilizing effect. (Refer to Appendix B in the 212-D100 Mitochondrial DNA Section Procedures Manual for the procedure.)

  All UV bulbs in a crosslinker will be replaced when the amount of time required to deliver 1 joule/cm^2 exceeds six minutes, or as necessary for individual bulbs due to outage.

- **WATER BATH:**

  Each Day of Use - Check and record temperature. Ensure the water level is at the correct height. If the temperature is greater than ± 2.0°C from the specified temperature the thermostat will be adjusted to obtain the correct temperature. The water bath temperature will be re-read and documented. If the correct temperature cannot be achieved the water bath will be removed from service until it can be repaired. The maintenance/repair will be documented on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

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  As Needed - Clean and replace water.

- **3500xL GENETIC ANALYZER:**

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  Annually - Have authorized vendor evaluate and perform necessary maintenance and repair. Record the service call on the "Equipment Calibration/Maintenance/Repair Form" found in Document 210-F600 or comparable log form.

  Refer to the Data Bank PowerPlex Fusion Procedures Manual, Appendix C for the maintenance of the Data Bank 3500xL Genetic Analyzers.

  Refer to Appendix H for the maintenance of the Casework 3500xL Genetic Analyzers.

  If a 3500xL is taken off line for repair/maintenance, prior to being put back in service, a performance check will be performed.

  - For nuclear DNA casework genetic analyzers, this check will consist of injecting a minimum of 2 ladders and 2 DNA samples of different known genotypes. The resulting data must be well-resolved, and the expected genotypes must be obtained.

  - For mitochondrial DNA casework genetic analyzer(s), this check will consist of injecting sequencing product from an HL-60 primer set amplification. The resulting sequences must have low background and peaks of high quality. Sequence analysis must yield accurate typing results.

2.2 Reagents, Chemicals and Supplies

A listing of commercial reagents, chemicals, supplies and laboratory prepared reagents needed for each procedure is found in the applicable Forensic Biology Procedures Manual containing that procedure.

Reagents and supplies which have passed the expiration date will not be used on casework or Data Bank samples. However, these reagents/supplies may be used on non-critical samples (i.e., training samples, research, etc.) and will be appropriately labeled as such.

When a reagent is diluted and used by a number of examiners and retained, a lot number must be created and the reagent must be traceable to the reagent log. The container with the diluted reagent must contain:

- The identity and concentration of the reagent
- the lot number (date of preparation followed by initials of preparer – see 2.2.3)
- the expiration date: Twelve months from date of preparation or reagent manufacturer's expiration date, whichever comes first

If a stock solution is diluted and used once and discarded or the dilution is used only by the examiner preparing the dilution, a new lot number does not need to be assigned or recorded in the reagent log. The original reagent stock lot number will be used; however, the dilution will expire twelve months from date of preparation or on the stock solution's expiration date, whichever comes first.

2.2.1 Sources of Reagents, Chemicals, and Supplies

All reagents and chemicals will be purchased from a manufacturer/vendor/supplier approved of by the Forensic Biology Program Manager or designee. Any changes in manufacturers/vendors/suppliers will be carefully checked by the Section Supervisor, Program Manager or designee to ensure the chemical being provided meets the specifications necessary for the reagent. Any changes in manufacturers/vendors/suppliers will be brought to the Forensic Biology Program manager's (Technical Leader's) or Assistant Technical Leader's attention so the following list can be updated as necessary.

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The following is a list of pre-approved manufacturers/vendors/suppliers:

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- Abacus Diagnostics
- Activate (bleach refills)
- Agilent
- Alfa-Aesor
- Applied Biosystems
- Beckman Coulter
- BT Sure
- Fisher
- Gilson
- Government Scientific Source
- Invitrogen
- Merck Millipore
- Molecular Bio Products
- Nalgene
- Orion
- Promega
- QIAGEN®
- Rainin
- RICCA
- Sarstedt
- Sigma-Aldrich (and its subsidiaries)
- ThermoFisher Scientific/Thermo Scientific
- USA Scientific
- VWR

2.2.2 Commercial Reagents, Chemicals and Supply Inventory

Upon receipt of all purchased reagents, chemicals and supplies, the packing slip will be checked for agreement with the items received. A copy of each packing slip initialed and dated by the receiver will be maintained. If the packing slip does not contain the lot number of the reagent or chemical, the individual inventorying the item will add the lot number to the packing slip. The date of receipt, date opened, date of verification (if appropriate) and initials of the person performing the verification and/or opening the reagent/standard will be marked on all incoming reagents/chemicals/supplies as well as the expiration date.

For commercial reagents without a supplied expiration date, a date of 3 years from the date of receipt will be used.

All chemicals and reagents will be stored according to the manufacturers' specifications and/or in a manner conforming to established safety requirements for each chemical/reagent. All chemicals containing biologicals will be disposed of in biohazard bags. Waste organic reagents will be placed in designated waste containers in a hood and removed from the laboratory by an appropriate means of disposal. All other reagents can be disposed of in the laboratory sink.

2.2.3 Laboratory Prepared Reagents and Solutions

All laboratory prepared reagents and solutions will be made with great care and using good laboratory practices.

All laboratory prepared reagents will be made with Type I water unless otherwise stated.

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All chemicals containing biologicals will be disposed of in biohazard bags. Waste organic reagents will be placed in designated waste containers in a hood and removed from the laboratory by an appropriate means of disposal.

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Concentrations preceding reagent components reflect the final concentrations of that specific component in the resulting mixture.

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The lot number assigned to laboratory prepared reagents/solutions will consist of the date prepared (two digit numerical representation of month, day and year) and the initials of the preparer.

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EXAMPLE: Lot number 100806TM represents a reagent/solution prepared on October 8, 2006 by Teresa Mathis.

All laboratory prepared reagents/solutions will be clearly labeled with the following:

- reagent/solution identity
- date of preparation
- expiration date (as specified in the Procedures Manual or labeled “no expiration”)
- the initials of preparer
- lot number
- storage requirements, if applicable
- date of autoclaving, if applicable

A log will be maintained for each laboratory prepared reagent/solution, including dilutions of laboratory concentrates except for personal use dilutions as outlined above. Each reagent/solution prepared will have the following information recorded in the log book:

- chemicals used
- commercial sources of chemicals
- lot numbers of chemicals
- date prepared
- initials of individual preparing reagent/solution
- expiration date
- other pertinent data, such as pH
- lot number

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2.2.4 Extraction Reagents

Reagents for which a recipe is listed in Appendix A of the FB PM Extraction of DNA may also be commercially purchased from an approved manufacturer/vendor/supplier, as applicable (see 2.2.1 of this manual).

Extraction reagents used for nuclear DNA extractions are routinely checked for reliability concurrent with testing. A quantitation result greater than N/A obtained for at least one sample associated with each type of extraction performed verifies that the reagents successfully released DNA. See FB PM Extraction of DNA, FB PM Interpretation of Fusion Data, and FB PM Interpretation of Yfiler™ Data for reagent blank requirements.

If all quantitation values for a particular extraction type are N/A, the sample types will be considered prior to deeming the verification of reliability to have failed. For example, a DNA IQ™ extraction set comprised solely of trace DNA samples with all quantitation values of N/A is not necessarily indication of failure of the reagents used. If an extraction set using the same lot numbers of reagents was or is shown to be successful, the reagents are reliable and no further action need be taken.

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In the case of a failure of the reagents to successfully release DNA (i.e., all quantitation values are N/A for a set of samples expected to give a quantitation result), re-extraction with different lot numbers will be performed, if possible

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2.2.5 Safety Data Sheets - SDS (formerly Material Safety Data Sheets – MSDS)

The SDS received from the manufacturer for each chemical used in the laboratory can be found in the designated SDS book or online. These data sheets will be kept current and readily available to all laboratory personnel.

2.2.6 Critical Reagents and Supplies

All critical reagents and supplies must be quality control tested for accurate, reliable performance prior to being used in the Section on casework or Data Bank samples. Refer to Section 3, Quality Control Of Critical Reagents and Supplies. The results of all quality control tests will be maintained in a ring binder or properly labeled file.

3 QUALITY CONTROL OF CRITICAL REAGENTS AND SUPPLIES

The following reagents/kits/supplies will be quality control tested as indicated below when a new lot is received into the laboratory. The successful completion of the quality control test is documented by the initials of the analyst reviewing the QC data on the paperwork and the placement of the quality control date on the reagent or packaging.

For the quality control of critical reagents and supplies used for mtDNA sequencing, see the Mitochondrial DNA Section Procedures Manual.

3.1 PCR-Based System Kits

3.1.1 Upon receipt and before use, each new lot of PCR-based kits will be quality control tested to ensure the integrity of the PCR reagents that are provided with the kit. **If the same lot of the PCR-based system kits is received for several shipments, it is not necessary to quality control test the kits again until a new lot is received into the laboratory.**

3.1.2 Amplify and type two samples that have been extracted of known DNA type, one negative amplification blank, and one positive amplification control per lot of new kits. For the AmpFℓSTR™ Yfiler™ Kit, the 9947A female control provided in the kit must also be amplified and typed using the most sensitive parameters used for casework samples (i.e., 5 µL amp, 2 µL/24 sec injection). Follow the amplification and typing procedures outlined in the applicable Forensic Biology Procedures Manuals or the Data Bank PowerPlex® Fusion Procedures Manual. **Record the loading position of the samples on the worksheets used for casework analysis or on the worksheets used for Data Bank analysis if the kit will be used on Data Bank samples only.**

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NOTE: In the Data Bank, the PowerPlex® Fusion kit may be utilized in a direct amplification protocol and the kit may also be utilized to amplify QIAGEN extracted DNA. Quality control verification must be performed for each protocol that the kit will be used for but not necessarily both protocols. For example, if the new lot of PowerPlex® Fusion kits will be used solely for direct amplification protocols, then it does not need to have a quality control verification performed with the extraction based protocol and vice versa.

3.1.3 Once the electropherograms have been analyzed, provided the expected types are obtained (i.e., known samples and positive amplification control) and the negative amplification blank does not produce results, the new lot of PCR- based kits will be considered to have passed the quality control verification and may be put on-line for routine use.

3.1.4 Once the new lot of PCR-based kits has passed the quality control verification, the kits will be labeled with the quality control date. Kits used for Data Bank samples will also be labeled with the protocol(s) that was used during the quality control verification. All worksheets and paperwork associated with the quality control test will be maintained in a ring binder or properly labeled file.

NOTE: This quality control test can be done in conjunction with the quality control testing of AmpliTaq™ Gold DNA polymerase and/or GeneScan™ 600 LIZ™ Size Standard, as applicable.

3.2 PunchSolution™ Kit

3.2.1 Each new lot of PunchSolution™ Kit will be quality control tested before use for the direct amplification of Data Bank samples. **If the same lot of PunchSolution™ Kit is received for several shipments, it is not necessary to quality control test the kit components again until a new lot number is received into the laboratory.**

3.2.2 Two previously typed samples, a reagent blank, a negative amplification control, and positive amplification control must be processed through the direct amplification method per lot of PunchSolution™ Kit. Follow the direct amplification and typing procedures outlined in the Data Bank PowerPlex® Fusion Procedures Manual. **Record the loading position of the samples on the worksheets used for Data Bank analysis.**

3.2.3 Once the electropherograms have been analyzed, provided the expected types are obtained for the known samples and positive control, and the reagent blank and negative amplification control do not produce results, the new lot of PunchSolution™ Kit will be considered to have passed the quality control verification and may be put on-line for routine use.

NOTE: This quality control test can be done in conjunction with the quality control testing of PCR based kits.

3.3 AmpliTaq™ Gold DNA Polymerase

3.3.1 Each new lot of AmpliTaq™ Gold DNA polymerase will be quality control tested before used on casework to verify the activity of the enzyme. **If the same lot of AmpliTaq™ Gold DNA polymerase is received for several shipments, it is not necessary to quality control test the AmpliTaq™ Gold DNA polymerase again until a new lot is received into the laboratory.**

3.3.2 Amplify and type two samples that have been extracted of known DNA type, one negative amplification blank, and one positive amplification control per lot of new AmpliTaq™ Gold DNA polymerase. Follow the amplification and typing procedures outlined in the applicable Forensic Biology Procedures Manuals. **Record the loading position of the samples on the worksheets used for casework analysis.**

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3.3.3 Once the electropherograms have been analyzed, provided the expected types are obtained for the known samples and positive control and the amplification blank does not produce results, the new lot of AmpliTaq™ Gold DNA polymerase will be considered to have passed the quality control verification and may be put on-line for routine use.

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3.3.4 Once the new lot of AmpliTaq™ Gold DNA polymerase has passed the quality control verification, the AmpliTaq™ Gold DNA polymerase stock box will be labeled with the quality control date. All worksheets and paperwork associated with the quality control test will be maintained in a ring binder or properly labeled file.

NOTE: This quality control test can be done in conjunction with the quality control testing of the PCR based kits.

3.4 Plexor® HY Kits

3.4.1 When a new lot number of the Plexor® HY System kit is received, it must be tested to determine if it conforms to the performance parameters defined by internal testing and the manufacturer's specifications prior to its use on casework.

3.4.2 A Plexor® HY System run will be performed, but it only needs to include two columns of the genomic standard; no additional DNA samples are required.

3.4.3 The slope, Y-intercept, R^2 and melt temperatures will all be assessed. Data points may be deselected, as described in the Forensic Biology Procedures Manual, Quantitation to improve the standard curve if necessary.

3.4.4 The data for the parameters listed above will be compared to the chart below to determine if they conform to within the indicated ranges.

3.4.5 If the data conform, then the quality control test is complete and the stock box or bag will be labeled with the quality control date. If not, the test will be repeated. If the data do not conform after the second attempt, then the manufacturer will be contacted to determine if that lot number should be replaced with a new lot, if additional testing needs to be performed, or if other corrective action needs to be implemented.

	Auto Average	Y Average
Slope	-3.81 ± 1.02	-3.56 ± 0.76
Y-intercept	24.92 ± 3.39	24.73 ± 2.84
R^2	0.99 ± 0.01	0.99 ± 0.01
Melt temperature	81.23 ± 1.5°C	82.59 ± 1.5°C

3.5 Standard Reference Material (SRM) Kit

The Standard Reference Material (SRM) kit or in-house traceable standards are designed to assure that the procedure for the DNA polymerase chain reaction (PCR) methodology is functioning properly and can be calibrated to the national standard provided by the National Institute of Standards and Technology (NIST).

3.5.1 Newly validated DNA methods (from amplification through characterization), typing test kits, or platform instrument models shall be checked against the NIST SRM or against NIST traceable samples prior to implementation of the method for forensic analysis. For STR analysis, a minimum of 2 samples with different DNA profiles (2 different male samples for Y STRs) will be carried from amplification through DNA typing. For mtDNA analysis, a minimum of one sample will be carried from amplification through sequencing. When substantial method changes/new validation has occurred for procedures that occur prior to amplification, traceable samples (a minimum of 2 with different DNA profiles for STR analysis or a minimum of one for mtDNA analysis) will be extracted and taken through DNA typing/sequencing, as applicable.

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3.5.2 Refer to Appendix I for information on how to prepare NIST traceable samples.

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3.5.3 Once the electropherograms have been analyzed, the expected types obtained and the controls typed appropriately, the laboratory's procedure will be considered to be functioning properly and calibrated to the national standard.

3.5.4 All worksheets and paperwork associated with the NIST SRM or NIST traceable check will be maintained with the associated validation paperwork.

3.6 p30 ABACARD®

3.6.1 Before using a new lot number of the ABAcard® p30 Test, its specificity must be tested and appropriately documented in the laboratory's quality control records.

3.6.2 The ABAcards® ("test devices") must be tested against human blood, vaginal fluid, saliva, feces, urine, a positive control (semen), and a negative control (buffer) to ensure that the test is working properly.

3.6.2.1 Samples of human blood, vaginal fluid, saliva, feces, urine, and semen will be prepared in-house. Samples should be air dried onto a swab or similar material prior to testing.

3.6.2.2 Label the known samples with the name of the substance (i.e., human semen, etc.) and the lot number (the date of preparation followed by the initials of the perparer).

3.6.2.3 Store known samples in the freezer.

3.6.2.4 It is also desirable to test dilutions of semen to determine the sensitivity of the test.

3.6.3 The quality control documentation will include (Refer to Document 210-F615):

- The lot number, receipt date, expiration date and manufacturer of the test devices
- The date of testing
- Initials of the person conducting the testing
- Results of the testing

3.6.4 Follow the testing procedure outlined in the Forensic Biology Procedures Manual, Evidence Screening and Collection of DNA Analysis for each of the QC samples being tested.

3.6.5 Once the appropriate tests have been performed on a lot number of the ABAcard® p30 Test, they need not be repeated for each case. If another shipment of the same lot number is received on a different date, they are also considered QC'd.

3.6.6 The test devices and buffer included in the kit are to be stored below 82° F (28° C). They are not to be frozen.

3.7 GeneScan™ 600 LIZ™ Size Standard

3.7.1 Each new lot of GeneScan™ 600 LIZ™ Size Standard will be quality control tested before use. **If the same lot of GeneScan™ 600 LIZ™ Size Standard is received for several shipments, it is not necessary to quality control test the GeneScan™ 600 LIZ™ Size Standard again until a new lot is received into the laboratory.**

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3.7.2 Amplify and type one negative amplification blank, and one positive amplification control per lot of new GeneScan™ 600 LIZ™ Size Standard. Follow the amplification and typing procedures outlined in the applicable Forensic Biology or Data Bank Procedures Manuals. **Record the loading position of the samples on the worksheets used for casework analysis or on the worksheets used for Data Bank analysis if the kit will be used on Data Bank samples only.**

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NOTE: If previously amplified controls are available, they may be used in lieu of amplifying a new set of controls.

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3.7.3 Once the electropherograms have been analyzed, provided the expected types are obtained for the positive control and the amplification blank does not produce results, the new lot of GeneScan™ 600 LIZ™ Size Standard will be considered to have passed the quality control verification and may be put on-line for routine use.

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3.7.4 Once the new lot of GeneScan™ 600 LIZ™ Size Standard has passed the quality control verification, the GeneScan™ 600 LIZ™ Size Standard box will be labeled with the quality control date. All worksheets and paperwork associated with the quality control test will be maintained in a ring binder or properly labeled file.

NOTE: This quality control test can be done in conjunction with the quality control testing of the PCR based kits.

3.8 WEN Internal Lane Standard 500

3.8.1 Each new lot of WEN Internal Lane Standard 500 will be quality control tested before use on casework if it is ordered and received as a separate component outside an amplification kit. **If the same lot of WEN Internal Lane Standard 500 is received for several shipments, it is not necessary to quality control test the WEN Internal Lane Standard 500 again until a new lot is received into the laboratory.**

3.8.2 Amplify and type one negative amplification blank, and one positive amplification control per lot of new WEN Internal Lane Standard 500. Follow the amplification and typing procedures outlined in the applicable Forensic Biology Procedures Manuals. **Record the loading position of the samples on the worksheets used for casework analysis or on the worksheets used for Data Bank analysis if the kit will be used on Data Bank samples only.**

NOTE: If previously amplified controls are available, they may be used in lieu of amplifying a new set of controls.

3.8.3 Once the electropherograms have been analyzed, provided the expected types are obtained for the positive control and the amplification blank does not produce results, the new lot of WEN Internal Lane

Standard 500 will be considered to have passed the quality control verification and may be put on-line for routine use.

3.8.4 Once the new lot of WEN Internal Lane Standard 500 has passed the quality control verification, the WEN Internal Lane Standard 500 box will be labeled with the quality control date. All worksheets and paperwork associated with the quality control test will be maintained in a ring binder or properly labeled file.

NOTE: This quality control test can be done in conjunction with the quality control testing of the PCR based kits.

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4 VALIDATION

The validation of new software, software changes, and new DNA technologies/methodologies utilized by the Section is addressed in this section. In addition, a summary of each validation study is maintained with the data. The summary and data are maintained by the Biology Program Manager as directed by the Department Quality Manual, Technical Procedures and Manuals. Summaries of applicable validation data are also available at each regional laboratory.

4.1 Requirements for Software Implementation/Changes

New software or new modules of existing software and modifications to software shall be evaluated in accordance with the FBI QAS to assess the suitability of the software for its intended use in the laboratory and to determine the necessity of validation studies or software testing. This evaluation shall include the determination of which studies will and will not be conducted and shall be documented.

4.2 Developmental Validation of PCR Based Analytical Procedures

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During the development of a DNA procedure and prior to the adoption of the procedure by the DNA laboratory, validation studies must have been conducted by the scientific community. These validation studies form the basis for evaluating the validity, accuracy, precision and reproducibility of a particular DNA analytical procedure. The developmental validation and scientific literature should include the following where applicable:

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- Characterization of the genetic marker
- Species specificity
- Sensitivity studies
- Stability studies
- Reproducibility
- Case type samples
- Population studies
- Mixture studies
- Precision and accuracy studies
- PCR based studies
  - PCR based studies include differential and preferential amplification, effects of multiplexing, assessment of appropriate controls and product detection studies.
- Peer reviewed publication of the underlying principles of a technology

4.3 Internal Validation of Established PCR Based Analytical Procedures

Prior to implementing a new DNA procedure, or an existing DNA procedure developed by another laboratory that meets the developmental criteria, the Section will validate the new PCR-based procedure internally to demonstrate the reliability of the procedure. This same prerequisite applies to any existing procedure to which significant modifications have been made. This validation forms the basis for assessing the specificity, reproducibility, and limitations of the particular PCR-based procedure. Prior to implementation the technical leader shall approve the internal validation studies. The internal validation shall include as appropriate the following:

- Known and non-probative evidence samples or mock evidence samples
- Reproducibility and precision studies
- Sensitivity and stochastic studies
- Mixture studies
- Contamination assessment
- NIST SRM or NIST SRM traceable check (see Section 3.5)

Regional labs may rely on internal validation conducted at another DFS lab but will need to complete the following validation studies:

- Precision
- Sensitivity

- Contamination assessment

Internal validation shall define quality assurance parameters and interpretation guidelines.

Before the PCR-based procedure is used by a casework examiner and/or Data Bank analyst, he/she will successfully complete a qualifying test.

A performance check shall be required if modifications are made to an instrument/software or if an additional critical instrument is received prior to implementation.

Significant software or instrument changes that may impact interpretation or the analytical process will require validation prior to implementation.

If a significant modification has been made to an analytical procedure, the modified procedure must be compared to the original using similar types of samples.

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5 INTERNAL CONTROLS AND STANDARDS – POLYMERASE CHAIN REACTION (PCR) PROCEDURES

Analysis of evidence samples will be conducted to provide the maximum information with the least consumption of the sample. This requires the use of critical judgment and the use of standards and controls. At each step of the testing procedure standards and controls must be used to evaluate the effectiveness of the testing process and to assure that the procedure is being properly performed. Refer to the Procedures Manual for the interpretation guidelines of the following controls.

5.1 Reagent Blanks

The reagent blank is a check for possible contamination of the reagents by human DNA. Testing of the reagent blank is performed by carrying out the DNA extraction in a tube containing no DNA, just reagents. This blank extract is then amplified and analyzed. Reagent blanks should produce no results.

The reagent blank is extracted and amplified concurrently with each extraction set; therefore, there will be at least one reagent blank for each type of extraction performed, including at least one for each fraction of a differential when performed. For any cases in which more than 50% of an evidentiary stain has been consumed, a devoted reagent blank (case reagent blank) will be processed with the respective case samples and dried and returned with the evidence extracts. Case reagent blanks will be processed/handled immediately following the associated case samples (i.e., interspersed throughout an extraction set for which there are multiple reagent blanks created).

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The reagent blank is extracted, amplified and typed with the same instrument model and the most sensitive volume and injection conditions as the extraction set.

- If multiple tubes are required to extract a stain, as detailed in the FB PM, Extraction of DNA, multiple tubes of reagent blank will be created that mimic the process through which the evidence is extracted.
- If multiple volumes are used within a set of samples extracted using a differential procedure, a separate reagent blank must be created for each volume used.
- If multiple volumes are used within a set of samples extracted using a non-differential procedure, either a single reagent blank using the highest volume used OR separate reagent blanks for each volume used must be created.

5.2 Random Sample (Used During Analysis of Convicted Offender and Arrestee Samples)

A random sample will be run with each set of convicted offender and arrestee samples to serve as a verification that the samples are successfully being entered into the Combined DNA Index System (CODIS) and the search algorithm is working properly. The random sample is a previously analyzed convicted offender sample for which the identity of the sample is known to the State CODIS Administrator or designee. This sample serves as an internal laboratory control and must be verified prior to the sizing data being considered acceptable. If a sample must be re-analyzed, a new random sample must be analyzed along with the sample.

5.3 Procedures for Estimating DNA Recovery

A procedure must be used for estimating the quantity of human DNA recovered from the evidence sample.

Refer to the Forensic Biology Procedures Manual, Quantitation for this procedure.

5.4 Negative Amplification Control (Negative Control)

The negative amplification control is a check of the PCR amplification reagents for contamination or for contamination that may have occurred during the set up of the PCR reaction. It is subjected to the most sensitive conditions of the sample set.

The negative amplification control is amplified concurrently with the sample set and typed with the same instrument model and the most sensitive volume and injection conditions as the sample set.

5.5 Positive Amplification Control (Positive Control)

The positive control is DNA of a known profile and is used to monitor the reagents used during the PCR reaction and the accuracy of the thermal cycler.

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6 DEDICATED WORK AREAS

To minimize the potential for carry-over contamination, the laboratory is organized so that the area in which amplified DNA is handled is physically isolated in a separate room from the work areas for DNA extraction and PCR setup. The laboratory has designated work areas for evidence handling, DNA extraction, and PCR setup. (These areas may be in the same room, but must be clearly delineated). Another area is reserved strictly for PCR amplification and typing, of casework samples and other activities that require handling of amplified DNA.

All functions performed during the processing and analysis of Data Bank samples are conducted in areas totally separate from the analysis of evidence samples. The Data Bank work areas, as defined below, are separate from the casework work areas. In addition, separate refrigerators/freezers from those used by the casework examiners will be used for storage of reagents and samples used by the Data Bank.

If contamination is identified which is not isolated to a single case and could adversely affect all of the DNA analyses conducted in the section, a supervisor will be notified immediately. Subsequently, the supervisor will notify the Biology Program Manager (Technical Leader) or Assistant Technical Leader of the contamination so that assistance can be provided to correct the problem and to ensure that the incident is properly documented. Depending on the level of contamination and severity, if necessary, the Section in the affected laboratory will be shut down until the problem can be corrected. In order to prevent compromising evidence samples, if the Biology Program Manager (Technical Leader) or Assistant Technical Leader is not available and therefore the problem cannot be discussed, if the level of contamination is wide spread, the supervisor has the discretion to shut down the Section to correct the problem once he/she has notified the Laboratory Director.

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6.1 DNA Extraction Work Area

This work area is used for evidence handling and for extraction and isolation of DNA. Microscopy, photography, and any other activities that involve the handling of evidence samples before amplification may also be performed in this work area.

If DNA extraction is performed robotically, refer to the Forensic Biology Procedures Manual, Biomek® NX^{P} Automation Workstation.

Equipment and supplies used in the DNA extraction work area may be exposed to relatively high concentrations of human genomic DNA. Therefore the dedicated equipment and supplies for this area should not be used in any other area except the DNA extraction work area.

6.2 PCR Setup Work Area

This work area is used for adding sample DNA to the PCR Reaction Mix.

If the PCR setup is performed robotically, refer to the appropriate Forensic Biology Procedures Manual.

To prevent transfer of exogenous human DNA into the PCR setup area, dedicated equipment and supplies used for PCR setup should not be taken out of the PCR setup work area.

6.3 Amplified DNA Work Area

This work area is a physically separate area used only for those activities that involve the handling of amplified DNA. This includes real-time qPCR quantitation and capillary electrophoresis of amplified DNA, waste disposal of amplified DNA solutions, and storage of amplified DNA.

Amplified DNA will be stored in a designated refrigerator or freezer in a separate box from the reagents used during the typing set up.

Amplified DNA or the dedicated equipment and supplies used to handle amplified DNA will not be removed from the amplified DNA work area. Exceptions for equipment needing service or calibration (pipettes, etc.) will be made only after a thorough cleaning/decontamination of the equipment.

Amplified DNA is handled carefully to reduce the possibility of dispersal. Gloves are always removed prior to leaving this area. A dedicated lab coat is worn whenever working with amplified DNA and removed prior to leaving this area.

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APPENDIX A – AUTOCLAVE QUALITY CONTROL

Due to the sensitive nature of DNA analysis, especially PCR applications, it is necessary to ensure adequate sterilization of glassware and solutions. Evaluation of the efficiency of sterilization is best accomplished by the utilization of a biological indicator. The spores of the thermophile *Geobacillus stearothermophilus ATCC 7953* grow during incubation at 55-60°C, but are inactivated if exposed to temperatures above 125°C. Acid production during the growth of the bacteria will cause a colorimetric change in the indicator solution from purple to yellow. It is necessary to use a positive control to show growth of the bacteria during normal incubation without inactivation by sterilization. Refer to package insert for storage instructions.

Precaution: The ampules contain live cultures. Handle with care to prevent breakage. Do not store in refrigerator with other biologicals. Ampules may not be used after expiration date. Use each ampule once and destroy by autoclaving or incineration after testing.

Biological Indicators for Steam Sterilization

Procedure:

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1 Label ampule(s) “Test” and “Control”.

2 Carefully place one purple ampule (Test) into the center of the autoclave.

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3 Proceed with sterilization as indicated by the manufacturer for a liquid solution or empty glassware.

4 After the cycle has ended, allow the ampule to cool for 10 to 30 minutes until it is cool enough to handle.

5 Following the manufacturer’s instructions, place the "Test" and "Control" ampules into a 56-60°C incubator or oven.

6 Observe ampules at 24 and 48 hours for color change from purple to yellow.

6.1 The "Control" ampule should change after approximately 24 hours of incubation. If the "Test" ampule changes within the 48 hour maximum, the sterilization cycle fails to meet the proper standard.

6.2 The sterilization cycle passes the test if the "Test" ampule does not turn yellow within the 48 hour incubation period but the "Control" ampule changes color.

7 Repeat test with new ampules if the "Control" does not change color from purple to yellow.

8 Record the results of the test. If the test fails, remove the autoclave from service and contact an authorized vendor to service the autoclave.

APPENDIX B – CALIBRATION OF THE BIOMEK®NXP AUTOMATION WORKSTATION

Calibration tests should be performed on a routine basis for the Biomek®NXP Automation Workstation. Home All Axes should be performed each day prior to use or whenever the robot has been turned off. The deck, including the STB1 ALP, should be framed once every three months or when needed. The deck framing provides coordinates to the software for each of the deck positions. The Accuframe tool is used to help perform this function.

1 Homing All Axes

1.1 If the robot was turned off and then turned on and a Home All Axes function is not performed, the software will prompt the user that it needs to be performed and will not allow the user to initiate a method.

1.2 Section 4.4 of the Extraction of DNA procedures manual provides the procedure for Homing All Axes.

2 Framing the Deck

2.1 The deck should be framed once every three months or whenever needed.

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2.2 Using the Instrument drop-down window, shown in Figure 1, select the Deck Editor.

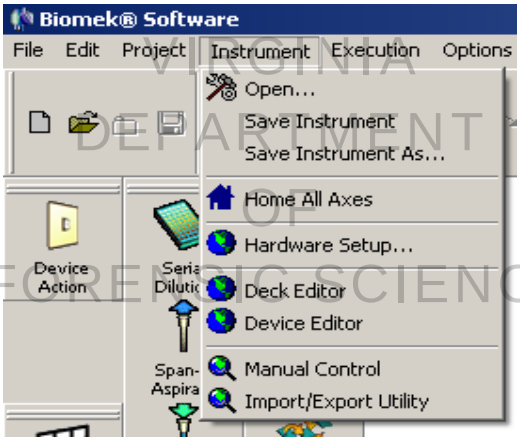

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Figure 1. Selecting the Deck Editor from the Instrument drop-down window.

2.3 The Deck Editor window will open up showing deck all of the deck positions (Figure 2). Positions 1-12 will be framed for the 4X3 ALP in the center of the deck.

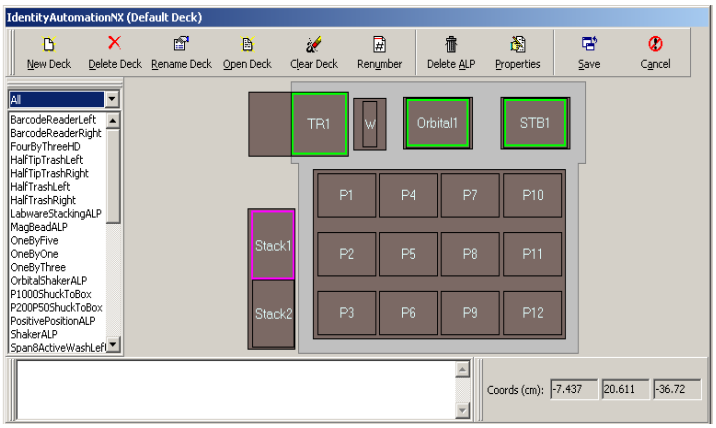

Figure 2. Deck positions.

2.4 Double-click on position P3 and the framing window will open (Figure 3). Depending on the position selected (e.g., P3 versus P10), the Auto Teach button will indicate which probe is to be used. The probe numbers begin at #1 in the back and end at #8 in the front-most position.

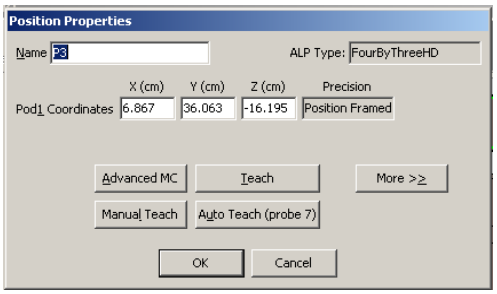

Figure 3. Position properties for framing.

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2.5 To prepare the probe for framing, unscrew the black mandrel surrounding the #7 probe (2nd probe from the front) and remove it. Next unscrew the silver probe, disconnect it from the system fluid tubing and remove it. Push the tubing up so that it is flush with end of the piece from which it protrudes. Next screw on the silver framing piece. It is asymmetrical, so it will only screw on in one orientation.

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2.6 Place the framing tool into the ALP position (#3), making sure that the Beckman Coulter and Accuframe logos are facing the front and the framing device is sitting flush in the ALP position.

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2.7 Click on the Auto Teach button (shown in Figure 3).

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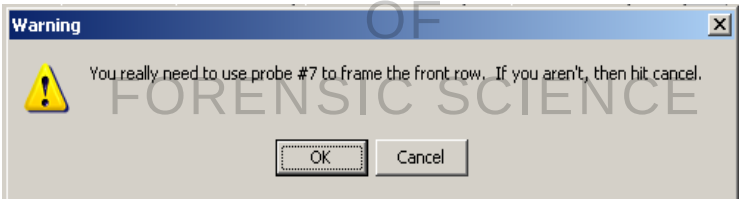

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Figure 4. Warning about the proper probe to use.

2.8 A window will pop up warning the user to use probe #7 for the deck framing at this position (Figure 4). After checking that probe #7 is being used, click OK.

2.9 Another warning will pop up, shown in Figure 5. After confirming that the distance moved will be tolerated, click OK and the framing will be performed.

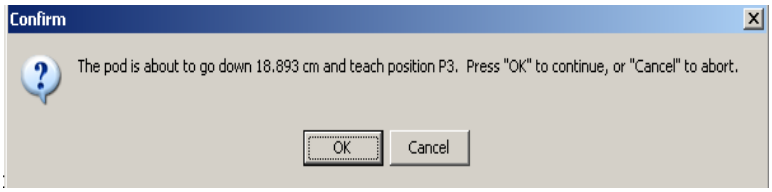

Figure 5. Confirm that the pod can move down in order to reach position P3.

2.10 After the framing is performed, another window will open up which provides the measured coordinates for the new framing as compared to the current coordinates (an example is shown in Figure 6).

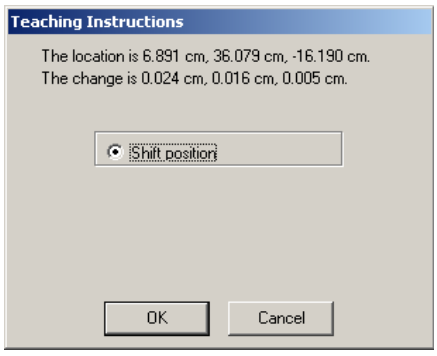

Figure 6. New framing coordinates.

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2.11 Framing coordinates are listed in the x, y and z-axes. **Cancel the changes unless one of the axes has a change greater than 0.025 cm.** If one of the coordinates displays a change greater than 0.025 cm, click OK.

2.12 Move the framing tool to the P12 position and repeat the process.

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2.13 For framing P10, the Orbital Shaker and the STB1, a different probe will be used. Remove the silver framing tool from the #7 probe position. Push the tubing back down so it protrudes and feed it onto the silver probe. Screw the probe into place and then screw the black mandrel back into position covering the probe. Remove the black mandrel from probe #1. Remove the silver probe and push the tubing back into the opening from which it protrudes. Screw the framing tool into the probe #1 position.

2.14 Repeat the process for framing but select the P10 position. Remove any device or piece of labware that might be placed into that ALP position prior to framing it. The window shown in Figure 7 will pop up. The Auto Teach button will now indicate that probe #1 should be used.

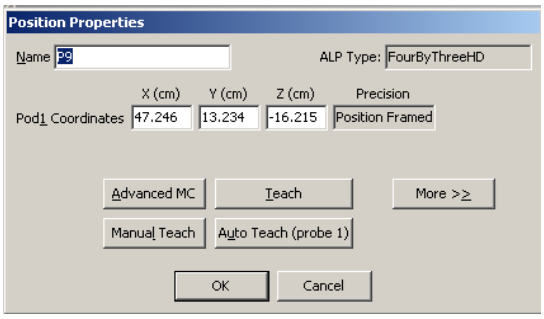

Figure 7. Position properties for framing.

2.15 Click on the Auto Teach button and follow the prompts as done previously for P3 and P12.

2.16 Click on Start. As directed in step 2.11 and in Figure 6, if the resulting values after framing are equal to or less than 0.025 at the x, y and z coordinates, then click Cancel. If one is greater than 0.025, then click OK.

2.17 Repeat the process for the STB1, going back to the deck screen shown in Figure 2 in order to double click on the STB1 ALP position.

2.18 Go through all of the same steps to frame the STB1 position. When the new coordinates have been measured, a window will pop up with the new coordinates, as shown in Figure 8.

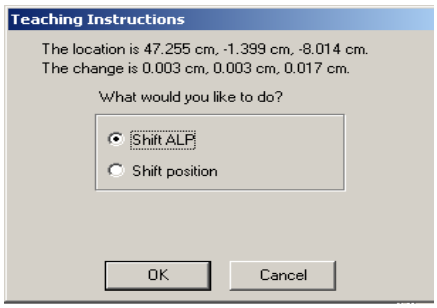

Figure 8. New framing coordinate for the STB1.

2.19 Again, unless the new coordinates are greater than 0.025 cm, then click cancel. If greater than 0.025, then either the Shift ALP or the Shift position can be checked before clicking OK.

2.20 Once the user returns to the deck layout, shown in Figures 2 and 9, the user will need to exit this prior to framing the orbital shaker. If any changes were made to the P1-12 or STB1 coordinates, then the user will need to click on the Save button otherwise the changes will not be saved. If no changes adopted for those deck positions, then click Cancel.

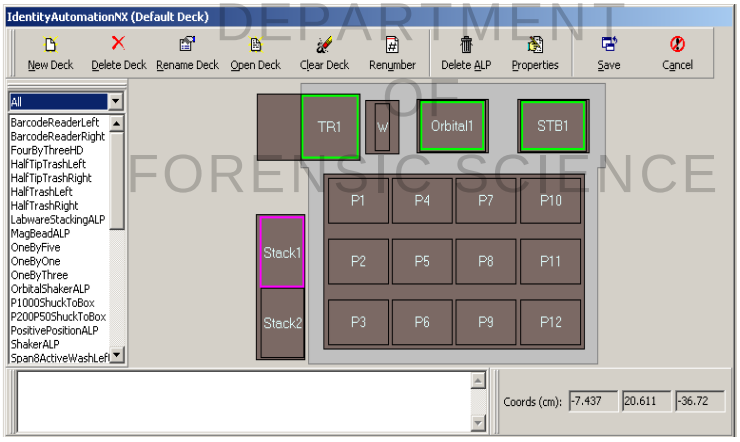

Figure 9. Deck layout.

2.21 The Orbital Shaker ALP should only be framed on an as needed basis (for e.g., if the Gripper is having trouble moving plates into it and this can't be remedied by adjusting the offsets). Move the Accuframe framing device to the Orbital Shaker ALP.

2.22 Under the Instrument drop-down window, select Deck Editor.

2.23 Double click on the Orbital Shaker ALP and follow the prompts as done previously for the other positions.

2.24 Frame the Orbital Shaker ALP. Once the window appears which provides the new coordinates, an example of which is shown in Figure 10, decide whether or not the new coordinates should be adopted. In the example shown in Figure 10, the y-coordinate is greater than 0.025 cm which would normally merit accepting the new coordinates, however, the y-axis coordinate was manually adjusted instead of adjusting an offset in order to accommodate plate movement onto the Orbital Shaker. Before accepting new coordinates for the Orbital Shaker, be certain that no manual adjustments to the framing coordinates were made.

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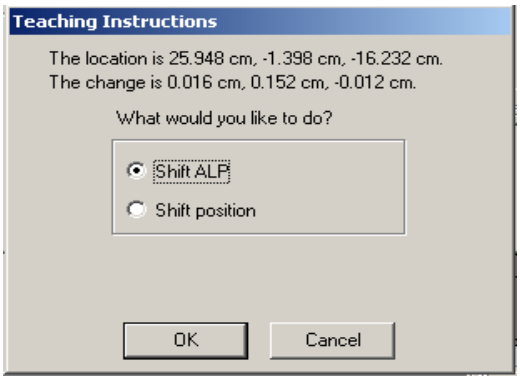

Figure 10. New coordinates for the Orbital Shaker ALP.

2.25 Click either OK to accept the new coordinates or Cancel. If new coordinates were accepted, then be certain to click Save when exiting the Deck Editor or the changes will be lost.

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NOTE: A test of plate movement and pipettor function must be performed if the Orbital Shaker is framed and changes to any of the axes are retained. Adjustments to the x, y or z offsets for a specific piece of labware may need to be made in order to move the plate properly and to pipette from the plate when sitting on the Orbital Shaker.

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Remove the silver framing tool from the probe #1 position, push the tubing back out, re-thread the silver probe #1 into the tubing and screw back into place. Follow that by screwing the mandrel back onto the probe.

APPENDIX C – CENTRIFUGE SPEED CHECK BY TACHOMETER

1 Upon receipt the calibration of each centrifuge will be checked by verifying the speed of the rotor. Once a year thereafter or following adjustments or repairs, the speed of the rotor will be checked.

2 The speed or number of rotations per minute (RPM) will be checked using a photoelectric tachometer. Use a high contrast label to mark a visibly accessible spot on the rotor. Follow the manufacturer's instructions provided with the tachometer to take the RPM readings. The speed of the centrifuge will be measured with the rotor installed and the cover closed. If the rotor speed is found to vary more than ± 1000 RPMs from the set speed of 10,000 RPMs, an authorized vendor will be called to recalibrate the speed of the centrifuge.

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APPENDIX D – CARE AND MAINTENANCE OF PIPETTES

1 Cleaning and Checking the Sealing System

Remove the tip ejector arm by pulling downward. Unscrew the shaft coupling slowly while holding the body and shaft firmly. The internal piston is spring loaded and will release when disengaged. Remove the white shaft from the piston assembly.

1.1 Shaft: Inspect the tip sealing end of the shaft for scratches, wear marks and splits.

1.2 Piston: Expose the piston surface. If the stainless steel surface is highly polished, the piston is in good condition and is not damaged. If the piston is highly stained or dirty, clean with isopropyl alcohol and a Kimwipe. Allow to air dry before reassembly. If the piston surface is scratched, heavily stained, corroded, or bent, the piston must be replaced.

1.3 Polyethylene Seal: Slide the white polyethylene seal up and down the piston. If the seal has resistance throughout the full length of the piston, it remains in good condition. If the resistance is spotty or non-existent, replace the seal. Seal replacement will not affect the pipette calibration.

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2 Routine In-House Verification of the Pipette Calibration

Once the pipette has been reassembled after cleaning, conduct a quick calibration check using graduated Rainin Pipette RT tips. Verify the pipetting volumes of the pipette as specified in the Pipette Verification Table below. The pipette tips are guaranteed to be accurate ± 3% at the specified reference lines. Should the pipette be visibly incorrect according to the reference line, the pipette will be sent to the manufacturer for repair.

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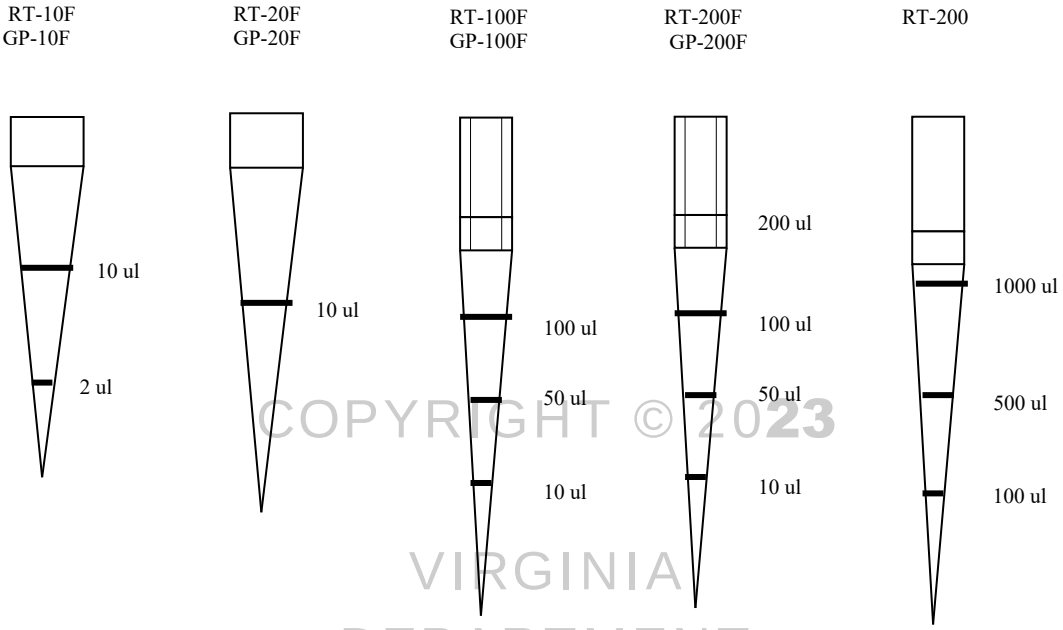
Pipette Verification Table

Pipette	Tip RT-10F		Tip RT-10F	Tip RT-20F	Tip RT-200F			Tip RT-200		
P-10	2 µL	10 µL								
P-20			2 µL	10 µL						
P-100					50 µL	100 µL				
P-200					50 µL	100 µL	200 µL			
P-1000								100 µL	500 µL	1000 µL



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APPENDIX E – PERFORMING THE STRATAGENE MX3005P™ INSTRUMENT QUALIFICATION TEST

1 Technical Notes

1.1 These instructions are adapted from that provided by the manufacturer in the Setup and User's Guide to the Stratagene Mx3005P™ QPCR System.

1.2 This test is a quality assurance measure to verify that the performance of the instrument is within factory specifications. The run takes approximately 2.5 hours to complete.

1.3 This test uses a preconfigured plate setup and thermal profile setup. No additional software setup steps are required. Do not modify the qualification test experiment or analyze data during the Qualification Test run.

1.4 Always wear gloves when handling the Qualification test plate, so as to ensure that no fingertip oils or other debris is transferred to the optical seal.

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2 Procedure for Performing the Stratagene Mx3005P™ Instrument Qualification Test

2.1 The qualification test plate can be obtained from Agilent.

2.2 Prepare the qualification test plate just prior to starting the run. Thaw the plate at room temperature for 15-30 minutes, leaving the plate sealed in the foil wrapper while thawing.

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2.3 After thawing, remove the foil wrapper and mix the contents of the wells by repeating this action five times:

2.3.1 Invert the plate. Use a sharp, swift, downward motion to move the liquid to the seal on top of each well. Observe the liquid to verify that the liquid has moved from the bottoms to the tops of the tubes.

2.3.2 Re-invert the plate, and use the same motion to return the liquid to the bottom of each well, again verifying liquid movement before proceeding.

2.4 After completing five mixing cycles, the liquid should be at the bottom of the wells. Briefly centrifuge the plate at 1000 rpm, if a plate centrifuge is available, to dislodge any bubbles and ensure all liquid is at the bottom of the wells. If no centrifuge is available, shake the liquid down to the bottom of the wells using the same motion described above. It is important to shake down any noticeable liquid on the tube seal. Small amounts of liquid spray retained on the tube sides should not affect test results.

2.5 Prior to loading the plate into the thermal cycler, ensure that no bubbles are present at the bottoms of the wells. Small bubbles near the liquid meniscus should not affect the test results, although bubbles at the bottom of the wells will interfere with the fluorescence readings.

2.6 Turn on the instrument and computer. Double click on the icon "Mx3005P Qualification Test" on the desktop of the computer controlling the Stratagene Mx3005P™ OR click the Instrument header, then Qualification Test within the MxPro software. Click "Next" (located in the bottom right corner of screen).

NOTES: When the MxPro software is started, if the "New Options" pop-up window appears, select Cancel.

Prior to starting the Qualification Test, select Filter Set Gain Settings under the instrument header and change the FAM setting to x8. Be sure to change this setting back to x2 when the Qualification Test run is complete.

2.7 Verify that the black "Perfect Fit" frame shipped with the instrument has been removed from the thermal block area. Load the plate in the thermal block, verifying the correct orientation (well A1 in the top, left corner).

NOTE: This opens much like a waffle iron – you should slide the black bar toward you and lift up. The plate should fit inside. The black top can then be placed over top of the plate and closed and latched to secure the plate.

2.8 Close the instrument door and click Start Run (located in the bottom right corner of the screen).

2.9 If the pre-run lamp warm-up dialog box appears, select Run Now. The system will begin the Instrument Qualification Test run.

NOTE: Do not open the instrument door during the run.

2.10 When the run is complete, the Instrument Qualification Test – Run complete dialog box will appear. Select Analyze. The software will open the Microsoft Excel software Instrument Qualification Validation template and will automatically export the Text Report data from the Instrument Qualification Test run into the Microsoft Excel software template, which has been preconfigured for data analysis.

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NOTE: If a "Server Busy" error dialog box appears, click Retry until the data export succeeds.

2.10.1 If any Norton anti-virus dialog boxes appear, select "Notify Again in 1 Day", if this option is available, and close the dialog box.

2.11 The Microsoft Excel software Validation template will open to the FAM analysis tab. First, verify that the data count cell reports the result "Good", ensuring that all of the data has been correctly imported into Microsoft Excel. Next, verify that the Result cell reports a Pass result. A Pass result indicated that the instrument is working properly. In this case, it is not necessary to email the data to Stratagene Technical Services. If the test reports a Check result, it is important to call Stratagene Technical Services (see product information sheet provided with the test plate for the current phone number) and send the three data files, including Customer Information.txt, into Stratagene technical Services by email for troubleshooting guidance.

2.12 After checking the results displayed on the Microsoft Excel software Validation template, go back into the MxPro software.

2.13 For a test report Check result only, the "Instrument Qualification Test – Email Experiment" dialog box will appear and present the option to automatically email the data or save the data to a disk to later email to Stratagene Technical Services (see product information sheet provided with the test plate for the current email address). Include a contact name, Virginia Department of Forensic Science, and the instrument serial number when sending the data to Technical services.

2.14 The "Instrument Qualification Test – Complete" dialog box appears, indicating that the Instrument Qualification Test is complete. Click Finish to conclude the test.

2.15 Record the results of the test in the appropriate QC log book.

APPENDIX F – THERMOMETER TEMPERATURE CALIBRATION

Thermometers (excluding NIST certified thermometers and those used to verify refrigerator and freezer temperatures) will be verified upon receipt for accuracy at the temperature of intended use. If available, a thermometer with a current certification from NIST should be used for comparison. However, a thermometer that is traceable to a NIST thermometer may also be used.

1 Tag each thermometer with an identifying number.

2 Place all of the thermometers in a vessel containing water at a temperature approximately 4°C below the temperature of intended use.

3 Record the temperature shown on each thermometer.

4 Next warm water to approximately 4°C above the temperature of intended use.

5 Place thermometers into the warmed water and record the temperature of each thermometer.

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6 Alternatively, for thermometers used in the high temperature range (i.e., heat blocks), the following method may be used:

6.1 Partially fill the wells of a heat block with sand OR partially fill a test tube with glass beads or glycerol and place it in a well in the heat block. Allow the sand, glass beads, or glycerol time to reach the temperature of the heat block. Place a NIST certified or NIST traceable thermometer in the sand in the heat block well or in the tube with the glass beads or glycerol. Record the temperature and repeat with the thermometer being calibrated.

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7 All thermometers should be within ± 1°C of the NIST thermometer or a thermometer traceable to a NIST thermometer.
- Any thermometer that varies more than ± 1°C in the range of intended use will not be used.

NOTE: If a thermometer is not acceptable at a specified temperature (e.g., 37°C, 56°C, 70°C, or 95°C) this does not preclude the thermometer from being used at another temperature providing it has passed the quality control test.

APPENDIX G – THERMAL CYCLER 9700 TEMPERATURE CALIBRATION, TEMPERATURE NON-UNIFORMITY, AND SYSTEM PERFORMANCE DIAGNOSTIC TESTS

1 DNA Thermal Cycler 9700 Temperature Calibration Verification Test

1.1 Verifying the Temperature Calibration

1.1.1 If the sample block heated cover is in the forward position, lift the lever, then slide the heated cover back.

1.1.2 Place the 9700 Temperature Verification Frame on the sample block.

1.1.3 Coat well A6 with mineral oil using a cotton swab.

1.1.4 Place the 0.2 mL probe assembly into well A6. Carefully thread the probe wire through the channel in the 9700 temperature verification frame to prevent damage to the probe and lead wires.

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1.1.5 Make sure the probe is connected to the digital thermometer and is seated properly. Close the cover carefully by sliding the heated cover forward, and pulling the lever down.

1.1.6 Turn on the digital thermometer and follow the instructions in the Temperature Verification Kit on how to operate the digital thermometer, Model 4500.

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1.1.7 Turn on the GeneAmp PCR System 9700. The main menu appears.

1.1.7.1 Select Utilities (F4)

1.1.7.2 Select Diagnostics (F1)

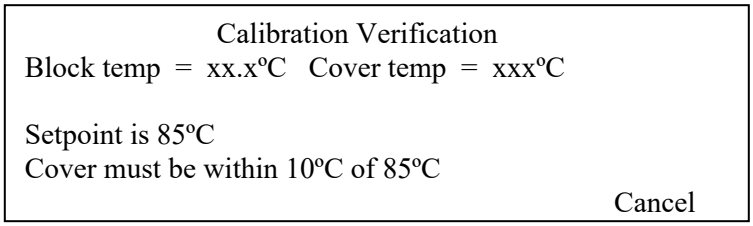
1.1.7.3 Select TmpVer (F3)

1.1.8 The Temperature Verification screen appears, select Temp (F1). This automatically configures the system 9700 for the Calibration Verification Test. The following screen appears:

Calibration Verification				
Block temp = xx.x°C Cover temp = xxx°C				
Place probe in well A6				
Run				Cancel
F1	F2	F3	F4	F5

1.1.9 Use the digital thermometer to take temperature readings of the sample well connected to the 0.2 mL Probe Assembly. You will take a reading at two different setpoint temperatures.

1.1.10 Select Run (F1) to start the Calibration Verification Test. The following screen appears with the setpoint values displayed.



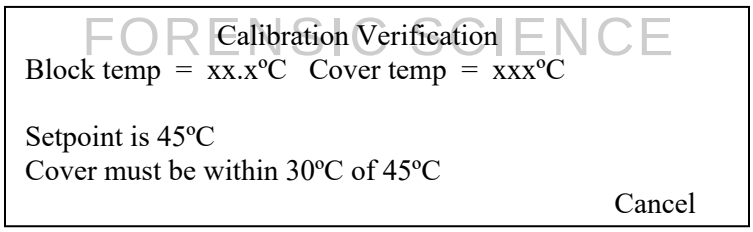
The cover must be within 1°C of 105°C. It may take several minutes for the system to ramp up.

1.1.11 The Calibration Verification screen counts down the time until the setpoint is reached. When the "Stabilization at setpoint" value decrements to zero, read the digital thermometer.

1.1.12 Using the numeric keys, type the value displayed on the digital thermometer in the "Enter actual block temperature" field displayed on the screen.

The digital thermometer displays a four-digit value; round this off to three digits before typing it in the Calibration Verification screen.

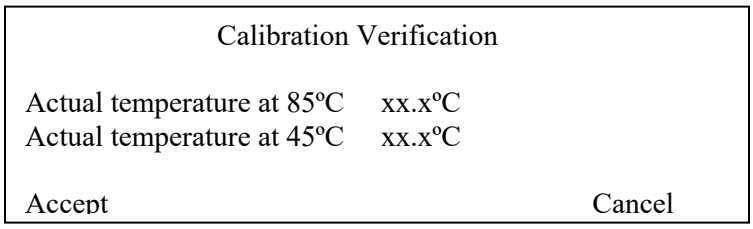
1.1.13 Press Enter on the keypad. The system automatically begins the second reading (45°C setpoint). The Calibration Verification screen appears with the setpoint value displayed.



The cover must be within 1°C of 105°C.

1.1.14 Repeat steps 1.1.11 and 1.1.12, then press Enter from the keypad.

1.1.15 The system 9700 evaluates the calibration of the sample block temperature for the setpoint values that were entered and displays the results. A summary screen appears at the conclusion of the test.



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1.1.16 Record the results on the Calibration Verification Test Data Sheet (Document 210-F601), then select Accept (F1).

1.1.17 If the sample block module is properly calibrated the Calibration Verification screen will display "Calibration is Good"

1.1.18 If the sample block module does not pass the Calibration Verification Test the screen will display "Instrument may require service. Contact Applied Biosystems Technical Support".

1.1.18.1 If the test fails, repeat the procedure to make sure the digital thermometer was not misread or that errors were not made entering data.

1.1.18.2 If the test fails again, contact Applied Biosystems Technical Support.

1.1.19 End the test by selecting Exit (F5).

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2 Temperature Non-Uniformity Test

2.1 Verify the temperature uniformity of the system 9700 with a 0.2 mL sample block module.

2.1.1 If the sample block heated cover is in the forward position, lift the lever, then slide the heated cover back.

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2.1.2 Place the 9700 Temperature Verification Frame on the sample block.

2.1.3 Coat wells A1, A12, C4, C9, F4, F9, H1, and H12 with mineral oil using a cotton swab.

2.1.4 Place the 0.2mL probe assembly into well A1. Carefully thread the probe wire through the channel in the 9700 temperature verification frame to prevent damage to the probe and lead wires.

2.1.5 Make sure the probe is connected to the digital thermometer and is seated properly. Close the cover carefully by sliding the heated cover forward, and pulling the lever down.

2.1.6 Turn on the digital thermometer and follow the instructions in the Temperature Verification Kit on how to operate the digital thermometer, Model 4500.

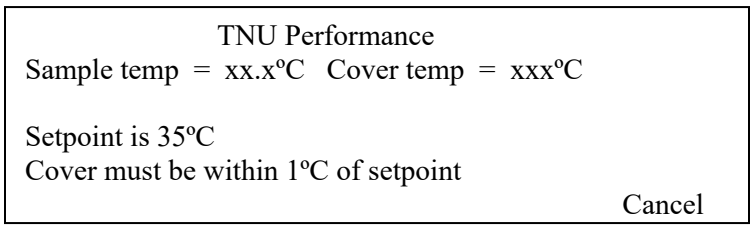
2.1.7 Turn on the GeneAmp PCR System 9700. The main menu appears:

2.1.7.1 Select Utilities (F4)

2.1.7.2 Select Diagnostics (F1)

2.1.7.3 Select TmpVer (F3)

2.1.8 The Temperature Verification screen appears, select TNU (F2). This automatically configures the system 9700 for the Temperature Non-Uniformity Test. The following screen appears:



It will take approximately 40 minutes for the cover temperature to reach the setpoint.

2.1.9 When the cover setpoint has been reached the following screen will appear:

```
                TNU Performance
Sample temp = xx.xºC  Cover temp = xxxºC

Place Probe in well A1
Press Run
Run                                  Cancel

   F1        F2        F3        F4        F5
```

2.1.10 Press Run (F1) and the following screen appears with the setpoint value displayed.

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```
                TNU Performance
Sample temp = xx.xºC  Cover temp = xxxºC

Setpoint is 37ºC
Sample must be within 1ºC of setpoint
                                     Cancel

   F1        F2        F3        F4        F5
```

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The sample block must be within 1.0°C of the setpoint and the cover must be within 1°C of 35°C. It may take a few minutes for the system to reach the setpoint temperature.

2.1.11 The TNU Performance screen counts down the time until the setpoint is stabilized. When the "Stabilization at setpoint" value decrements to zero, read the digital thermometer.

2.1.12 Using the numeric keys, type the value displayed on the digital thermometer in the "Enter actual block temperature" field displayed on the screen.

The digital thermometer displays a four-digit value; round this off to three digits before typing it in the Calibration Verification screen.

2.1.13 Press enter on the keypad. The TNU Performance screen appears with setpoint value displayed at 94°C. When the sample temp reaches 94°C the setpoint value changes to 37°C and the following screen appears.

```
                TNU Performance
Sample temp = xx.xºC  Cover temp = xxxºC

Place probe in well A12
Press Run
Run                                  Cancel

   F1        F2        F3        F4        F5
```

During the 94°C setpoint test this will be reversed. The first setpoint value displayed is 37°C, when this sample temp setpoint is reached the setpoint value changes to 94°C and the TNU Performance screen appears.

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2.1.14 Slide the heated cover back and place the probe in A12. Make sure the probe is seated properly. Close the cover carefully by sliding the heated cover forward, and pulling the lever down. Repeat steps 2.1.10 through 2.1.13. Complete the steps for all 8 wells: A1, A12, C4, C9, F4, F9, H1, and H12.

2.1.15 After reading the last well (H12) press Enter and the 9700 system will begin the second reading (94°C setpoint). The TNU Performance screen appears with the setpoint displayed at 94°C the cover temp will increase and must be within 1°C of 105°C. It may take a few minutes for the system to stabilize at the setpoint temperature.

2.1.16 When the system has stabilized the TNU Performance screen with the "place probe in well A1" prompt appears. Repeat steps 2.1.9 to 2.1.13.

2.1.17 After the last reading for setpoint 94°C is completed, the system 9700 evaluates the uniformity of the sample block temperature for the setpoint values that were entered and displays the results. A summary screen appears at the conclusion of the test.

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Well	94ºC	37ºC	Well	94ºC	37ºC
A1	xx.x	xx.x	F4	xx.x	xx.x
A12	xx.x	xx.x	F9	xx.x	xx.x
C4	xx.x	xx.x	H1	xx.x	xx.x
C9	xx.x	xx.x	H12	xx.x	xx.x
Accept			More		Cancel

F1 F2 F3 F4 F5

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2.1.18 Record the results on the Temperature Non-Uniformity Test Data Sheet (Document 210-F601), then select Accept (F1).

2.1.19 When the system completes the Temperature Non-uniformity test, the TNU Performance screen appears with either "Pass" or "Fail" after each setpoint.

2.1.19.1 If the temperature of the sample block wells is uniform then the test has passed.

2.1.19.2 If the temperature variation of the sample block wells exceeds performance expectations the test has failed.

2.1.19.2.1 If the test fails, repeat the procedure to make sure the digital thermometer was not misread or that errors were not made entering data.

2.1.19.2.2 If the test fails again, contact Applied Biosystems Technical Support.

2.1.20 End the test by selecting Cancel (F5).

2.1.21 If all measurements are completed, remove the probe and the Temperature Verification Frame from the sample block and turn off the digital thermometer.

2.1.22 Clean the wells of the sample block using cotton swabs.

3 System Performance Diagnostics: Rate Test and Cycle Test

3.1 Testing the integrity of the cooling and heating system

3.1.1 Before beginning the tests, an empty 96-well plate with full plate cover or caps must be in the sample block.

3.1.2 Turn on the GeneAmp PCR System 9700. The main menu appears.

3.1.2.1 Select Utilities (F4)

3.1.2.2 Select Diagnostics (F1)

3.1.2.3 Select System (F2)

3.1.3 The System Performance Screen appears

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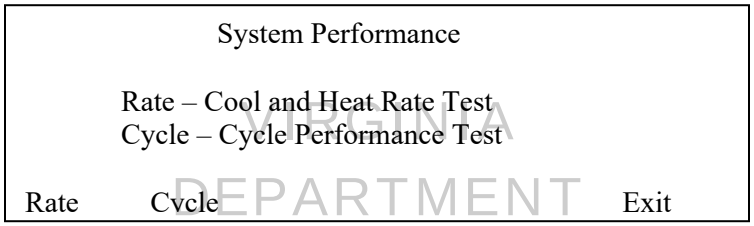

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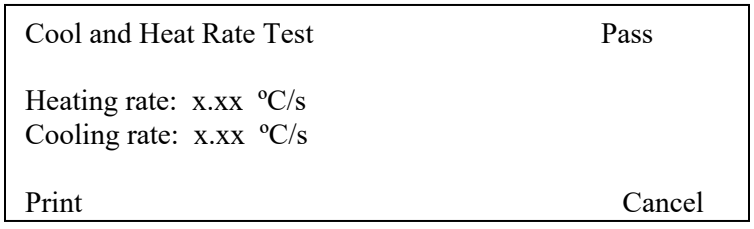
3.1.4 Select Rate Test (F1) to verify that the Peltier units are operating correctly. The System Performance screen displays the following message: "WARNING!! Install the appropriate empty consumables into the sample block. Refer to System Performance Section of the Block User Manual."

3.1.5 Select Cont. (F1) after the plate and cover has been installed and the Cool and Heat test screen appears.

3.1.6 The instrument then runs through a series of tests where the sample block ramps to and stabilizes at 35°C, 94°C and 4°C, which is displayed on the screen. At the conclusion of the test, the Cool and Heat screen appears displaying the results and whether the test results passed or failed. Record this information in the Cool and Heat Rate Test area on the worksheet (Document 210-F601). If the test fails contact Applied Biosystems Technical Support.



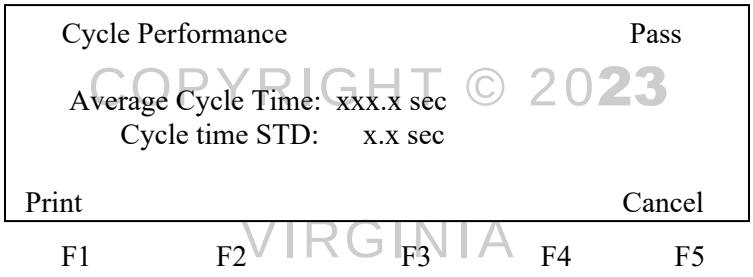
3.1.7 The following table lists the passing ranges for the Rate test:

	Heating Rate	**Cooling Rate**
Gold 96-well	3.0 to 6.0 ºC/second	3.0 to 6.0 ºC/second
Aluminum 96-well	1.5 to 2.5 ºC/second	1.4 to 2.5 ºC/second

3.1.8 Return to the System Performance screen by selecting Cancel (F5) and select Cycle Test (F2) to verify that the PCR cycling function operates properly. The System Performance screen displays the following message: "WARNING!! Install the appropriate empty consumables into the sample block. Refer to System Performance Section of the Block User Manual."

3.1.9 Select Cont. (F1) after the plate and cover has been installed.

3.1.10 The Cycle Test executes a standard PCR cycle reaction (6 cycles), measures, and reports the average cycle time, and the cycle to cycle variation. At the conclusion of the test, the Cycle Test screen will display the results and whether the test results passed or failed. Record this information in the Cycle Performance area on the worksheet (Document 210-F601). If the test fails contact Applied Biosystems Technical Support.



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3.1.11 The following table lists the passing ranges for the Cycle Test.

Average Cycle Time <= 160 sec
Cycle Time STD < 5 sec

3.1.12 Select Cancel (F5) to exit the test.

APPENDIX H – MAINTENANCE OF THE CASEWORK 3500xL GENETIC ANALYZERS

1 Maintenance requirements for the 3500xL

Task	Frequency
Restart the computer and instrument	At minimum – before a casework run if last restart was >14 days prior
Run the Wash Pump and Channels Wizard (conditioning reagent – Promega Corp)	Monthly or as needed
Flush the water trap (pump trap)	Monthly or as needed
Empty the water trap waste container. The waste container is to the right of the pump block	As needed
Change polymer pouch (replenish polymer)	At minimum - before a casework run if current pouch was installed >14 days prior
Change anode and cathode buffer cartridges	At minimum - before a casework run if current cartridges were installed >14 days prior
Replace cathode buffer septa	In conjunction with replacement of cathode buffer cartridge
Clean the autosampler and drip tray	As needed
Install a new capillary array	As needed based solely upon data obtained– for ex.: if poor sizing precision or allele calling results are noted; if poor resolution and/or decreased signal intensity is observed

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2 Wizards

Wizards are automated instrument processes which allow the user to perform a variety of tasks. Each wizard has been designed with specific instructions to achieve the purpose of the wizard. The wizards are accessed by selecting the desired wizard from the “Maintenance Wizards” menu and include:

- Install a Capillary Array – used to install or replace a capillary array – takes 15-45 minutes
- Remove bubbles from the polymer pump – used to remove bubbles in the polymer pump fluid path from the polymer pouch through the pump, array port and anode buffer container – takes 5-15 minutes
- Wash the pump chamber and channels – uses conditioning reagent to prime the polymer pump, wash the polymer pump between polymer type changes and during instrument shut down (each pouch is adequate for a one-time use) – takes about 40 minutes

- Record the date of the conditioning reagent run, lot number and expiration date of the reagent pouch in the appropriate QC log.

- Fill the array with fresh polymer – fills the array with fresh polymer

- Replenish the Polymer Installed on the Instrument – for replacing the polymer pouch on the instrument with a new pouch of the same type of polymer – takes 10-20 minutes

  - The new polymer pouch should be allowed to equilibrate to room temperature prior to installation.

- Change the type of polymer installed on the instrument with the option to change arrays – allows the user to change the type of polymer on the instrument (POP-4 to POP-6, for example).

- Shutdown the instrument – for both long-term and short-term shutdown – takes 60 minutes

3 Restarting the computer and instrument

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3.1 Shutting down the instrument and computer

3.1.1 Close the Data Collection Software.

3.1.2 Shut the instrument off.

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3.1.3 Shut the computer down.

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3.2 Starting the instrument and computer

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3.2.1 Turn on the computer, but do NOT log on.

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3.2.2 Power on the instrument and wait for the solid green light.

3.2.3 Log onto the computer and wait until the “3500 Services Loaded” at the lower right corner of the screen is completely loaded and indicated with a green check mark.

3.2.4 Launch the Data Collection Software by double-clicking on the 3500 instrument icon.

4 Changing the Anode Buffer Container (ABC)

4.1 Verify that the buffer level is at or above the fill line and check that the seal is intact.

4.2 Invert the ABC and tilt it slightly to make sure most of the buffer is in the larger side of the container. Less than 1 mL should remain in the smaller side of the container.

4.3 Peel off the seal at the top of the ABC and place the ABC into the anode end of the instrument.

4.4 Click Refresh on the dashboard to update the screen.

5 Changing the Cathode Buffer Container (CBC)

5.1 Press the Tray button on the instrument to bring the old CBC forward and discard it.

5.2 Verify that the buffer level of the new CBC is at or above the fill line and check that the seal is intact.

5.3 Tilt the CBC back and forth gently to ensure the buffer is evenly distributed across the top of the baffles.

5.4 Peel off the seal at the top of the CBC and wipe off any buffer on top of the CBC to make sure the container is dry.

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5.5 Align and place the appropriate new septa on both sides. Push the septa lightly into the holes to start and then push firmly to seat the septa. Install the CBC on the autosampler.

5.6 Close the instrument door, wait for the green light and click Refresh on the dashboard to update the screen.

6 Flush the water trap (pump trap)

6.1 Fill a Luer lock syringe with a capacity of at least 20 mL with Type I water. Expel bubbles from the syringe. Do not use a syringe smaller than 20mL, as the pressure generated will be too great.

6.2 Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting with the other hand.

6.3 Open the Luer fitting by grasping the body of the fitting and turning it to loosen. Turn counterclockwise approximately one-half turn.

6.4 DO NOT USE EXCESSIVE FORCE when you push the syringe plunger as this may damage the trap seals. Take approximately 30 seconds to flush 5 mL of water through the trap.

6.5 Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.

6.6 Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

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7 Performing a Spatial Calibration

7.1 A spatial calibration is used by the instrument to correlate the signal from each capillary with the signal detected by the CCD camera and MUST be performed after any of the following events:

- The capillary array has been replaced
- The detector door has been opened or the detection cell has been moved
- The instrument has been moved
- A service engineer has performed an optical service procedure, such as a realignment or replacement of the laser or CCD camera or mirrors on the instrument

7.2 To access the Spatial Calibration screen, select Maintenance→ Spatial Calibration in the navigation pane.

7.3 Select “No Fill” or “Fill” which will either not fill the array with fresh polymer before starting the calibration or fill the array with fresh polymer before starting the calibration.

7.4 Check “Perform QC checks”. The software will check each capillary against the specified range for spacing and intensity.

7.5 Click Start Calibration. The display updates as the run progresses.

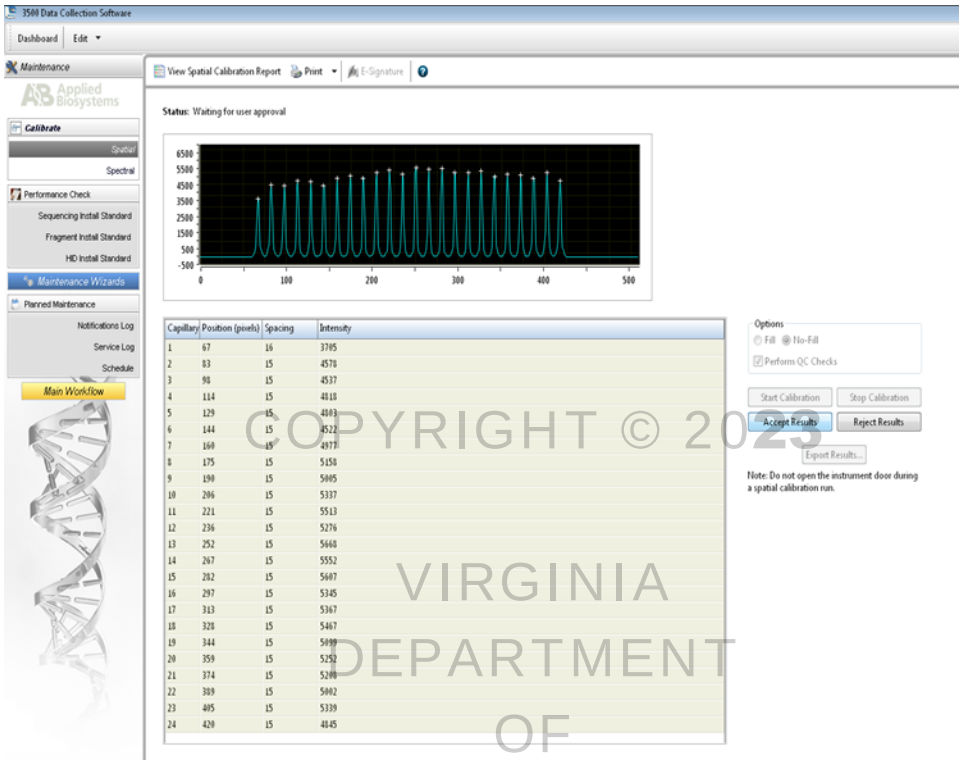
7.6 A Spatial QC Check error message is displayed if:

- The average peak height or individual peak height is below the threshold
- Uniformity or capillary spacing exceeds the threshold

7.7 When the run is complete, evaluate the calibration profile to ensure that you see:

- One sharp peak for each capillary – small shoulders are acceptable.
- One marker (+) at the apex of every peak – no off-apex markers.
- An even peak profile (all peaks about the same height)

7.8 If the results meet the above criteria, click Accept Results. An example of a successful spatial profile is shown below.



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7.9 If the results do not meet the above criteria, click Reject Results.

7.10 The spatial can be re-run. If problems persist, refer to the "Spatial Calibration Troubleshooting" section in the Applied Biosystems 3500xL Genetic Analyzer user guide.

NOTE: The software does not save historical calibrations. Only the most recent spatial calibration results are maintained.

8 Performing a Spectral Calibration

8.1 Both PowerPlex® Fusion and AmpFℓSTR™ Yfiler™ use dye set G5. A G5 spectral performed using Promega's spectral kit will not perform well with Thermo Fisher's Yfiler™ kit and vice versa. If both kits will be used on the same 3500xL instrument, then a differently defined G5 spectral should be created for each amplification kit. See 8.8 for the preparation of the matrix standard for PowerPlex® Fusion and 8.9 for the preparation of the matrix standard for AmpFℓSTR™ Yfiler™.

The BigDye® Terminator v1.1 Cycle Sequencing Kit uses dye set E. See 8.10 for the preparation of the matrix standard for the BigDye® Terminator kit.

8.2 Generating a matrix using Dye Set G5

8.2.1 Create a dye set (proceed to 8.4 if the dye set has previously been created).

8.2.2 In the Library under the Analyze menu on the left, select Dye Sets and Create.

8.2.3 In the Create Dye Set window, name the dye set (e.g., Promega _G5_WEN), select Matrix Standard as the chemistry. For a Fusion Dye Set, under Parameters, change the after scan value to 800 and Save.

8.2.4 Once generated, this file is applied during sample detection to calculate the spectral overlap between the five different dyes and separate the raw fluorescent signals into individual dye signals.

8.3 Generating a matrix using Dye Set E

8.3.1 Create a dye set (proceed to 8.4 if the dye set has previously been created).

8.3.2 In the Library under the Analyze menu on the left, select Dye Sets and Create.

8.3.3 In the Create Dye Set window, name the dye set (e.g., E), select Matrix Standard as the chemistry. Under Parameters, change the after scan value to 1500, if not the default, and Save.

8.4 Perform a spectral for each dye set/polymer type combination you will use. A spectral must also be performed after any of following events:

- The capillary array has been replaced
  NOTE: When performing a spectral calibration after an array replacement, two to three formamide injections can be run prior to the spectral, if desired.
- A decrease is seen in spectral separation (pull-up in peaks) in the raw or analyzed data
- A service engineer has performed an optical service procedure, such as a realignment or replacement of the laser or CCD camera or mirrors on the instrument

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8.5 If not already done so, perform a spatial calibration.

8.6 Pre-heat the oven.

- 60°C for PowerPlex® Fusion or AmpFℓSTR™ Yfiler™ (POP-4)
- 55°C for BigDye® Terminator v1.1 Cycle Sequencing Kit (POP-6)

This is very important – preheating mitigates subtle first-run migration rate effects.

8.7 Check the consumables status on the dashboard to ensure they are not expired and that adequate injections remain.

8.8 Preparation of the matrix standard for PowerPlex® Fusion (Use PowerPlex® 5C Matrix Standard – Promega Corp.)

8.8.1 Remove the 5C Matrix Mix and one tube of Matrix Dilution Buffer from the freezer, thaw and vortex to mix thoroughly. DO NOT centrifuge.

NOTE: The 5C Matrix Mix should be stored at 2-10°C after first use.

8.8.2 Add 10 µL of the 5C Matrix Mix to the tube of Matrix Dilution Buffer. Vortex to mix thoroughly. DO NOT centrifuge.

NOTE: The diluted 5C Matrix Mix can be stored at 2-10°C for up to one week.

8.8.3 Add 10 µL of the diluted 5C Matrix Mix to 500 µL Hi-Di formamide and vortex to mix thoroughly. DO NOT centrifuge.

8.8.4 Add 15 µL of the formamide/dye mix to each well of a three column set of wells (columns 1-3, 4-6, 7-9 or 10-12) in a 96 well plate.

8.8.5 After placing the septa on the plate, briefly centrifuge to remove bubbles.

8.8.6 DO NOT denature the samples.

8.8.7 Place the plate in the plate base and cover with a plate retainer.

8.8.8 Place the plate in the autosampler making sure to designate in the software whether position A or B is chosen.

8.8.9 To perform the spectral, click on the Maintenance tab and select Spectral under the Calibrate menu on the left. From the Chemistry Standard drop-down menu, choose Matrix Standard, choose the Dye Set previously created in 8.2 from the Dye Set drop-down menu and select the starting well. Verify that "Allow Borrowing" and "Perform Run 2/Run 3 if Run 1 fails" are checked. Click Start Run.

8.9 Preparation of the matrix standard for AmpFℓSTR™ Yfiler™ (Use DS-33 Matrix Standard Dye Set G5 – Thermo Fisher Scientific.)

8.9.1 Remove a tube of the matrix standard from the refrigerator and vortex thoroughly to mix. Briefly centrifuge.

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8.9.2 Follow the package insert for Hi-Di™ formamide:matrix standard ratios (245 µL Hi-Di™ + 5 µL DS-33 matrix standard).

8.9.3 Vortex the formamide/matrix standard mixture thoroughly. Briefly centrifuge.

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8.9.4 Add 10 µL of the formamide/dye mix to each well of the first three columns (or 1 full injection) of a 96-well plate.

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8.9.5 After placing the septa on the plate, briefly centrifuge to remove bubbles.

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8.9.6 Denature the samples at 95 °C for 3 minutes, then immediately snap cool the plate in a freezer block for 3 minutes.

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8.9.7 Place the plate in a plate base and cover with a plate retainer.

8.9.8 Place the plate in the autosampler making sure to designate in the software whether position A or B is used.

8.9.9 To perform the spectral, click on the Maintenance tab and select Spectral under the Calibrate menu on the left. From the Chemistry Standard drop-down menu, choose Matrix Standard, choose the Dye Set previously created in 8.2 from the Dye Set drop-down menu and select the starting well. Verify that "Allow Borrowing" and "Perform Run 2/Run 3 if Run 1 fails" are checked. Click Start Run.

8.10 Preparation of the matrix standard for BigDye® Terminator v1.1 Cycle Sequencing Kit (Use BigDye® Terminator v1.1 sequencing standard.)

8.10.1 Briefly centrifuge the BigDye® Terminator v1.1 sequencing standard to collect its contents.

8.10.2 Add 300 µL of Hi-Di formamide to resuspend the sequencing standard.

8.10.3 Thoroughly mix the contents of the sequencing standard tube and spin briefly in a centrifuge.

8.10.4 Dispense 10 µL of the sequencing standard into wells A1-H3 of a 96-well reaction plate.

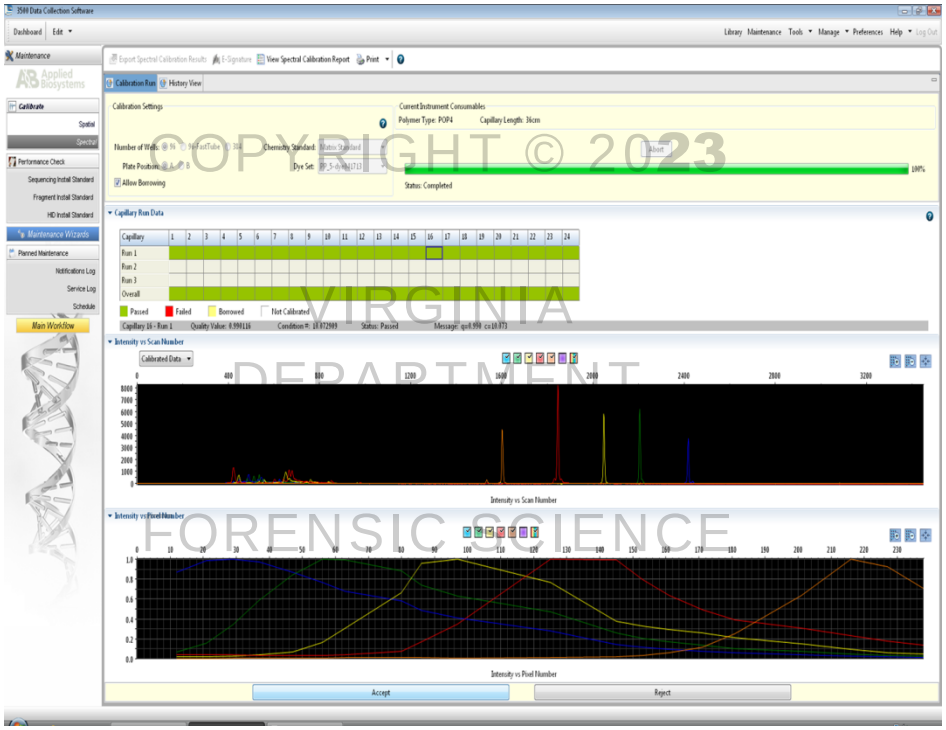
8.10.5 Cap the wells and denature at 95 °C for 2 minutes followed by a brief spin in a centrifuge to ensure there are no bubbles in the bottom of the wells.

8.10.6 Remove caps and assemble the plate assembly. Place the plate in the autosampler making sure to designate in the software whether position A or B is used.

8.10.7 To perform the spectral, click on the Maintenance tab and select Spectral under the Calibrate menu on the left. From the Chemistry drop-down menu, choose Sequencing Standard and choose Dye Set E. Verify that "Allow Borrowing" and "Perform Run 2/Run 3 if Run 1 fails" are checked. Click Start Run.

8.11 Evaluating the Spectral Calibration run for nuclear STR kits

8.11.1 The figure below shows a complete and successful spectral run for Dye Set G5_Promega.



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8.11.2 In the Capillary Run Data field, a passing capillary is shown in green, a failed capillary is shown in red and a capillary for which adjacent data was borrowed is shown in yellow.

8.11.3 To display the results for an individual capillary (spectral data, Quality Value and Condition Number), click on the associated colored box in the Capillary Run Data field.

Quality Value – reflects the confidence that the individual dye emission signals can be separated from the overall measured fluorescent signal. It is a measure of the consistency between the final matrix and the data from which it was computed. A Quality Value of 1.0 indicates high consistency, providing an ideal matrix with no detected pull-up/pull-down peaks.

Condition Number – indicates the amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set. If there is no overlap in a dye set, the Condition Number is 1.0 (ideal), the lowest possible value. The condition number increases with increasing peak overlap.

8.11.4 Examine the data for all capillaries before accepting the spectral calibration results to be sure they each meet the following requirements:

- Quality Value minimum – 0.95

- Condition Number maximum – 13.5
- Order of peaks in spectral profile from left to right should be:

  Blue → green → yellow → red → orange
- Peak morphology in the spectral profile – peaks are separate and distinct with no gross overlaps, dips, or irregularities.
- Order of peaks in the raw data profile from left to right should be:

  Orange → red → yellow → green → blue
- No extraneous peaks in the raw data should appear.
- No excessive pull-up in the calibrated data (e.g., large yellow peak under red peak) should appear.

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8.11.5 If any of the above criteria are not met, the calibration has failed.

8.11.5.1 The spectral calibration can be re-run. If problems persist, refer to the "Spectral Calibration Troubleshooting" section in the Applied Biosystems 3500xL Genetic Analyzer user guide.

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8.12 Evaluating the Spectral Calibration for mitochondrial DNA sequencing

8.12.1 The figure below shows a complete and successful spectral run for Dye Set E.

8.12.2 In the Capillary Run Data field, a passing capillary is shown in green, a failed capillary is shown in red, and a capillary for which adjacent data was borrowed is shown in yellow.

8.12.3 To display the results shown for an individual capillary (spectral data, Quality Value, and Condition Number), click on the associated colored box in the Capillary Run Data field.

Quality Value – reflects the confidence that the individual dye emission signals can be separated from the overall measured fluorescent signal. It is a measure of the consistency between the final matrix and the data from which it was computed. A Quality Value of 1.0 indicates high consistency, providing an ideal matrix with no detected pull-up/pull-down peaks.

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Condition Number – indicates the amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set. If there is no overlap in a dye set, the Condition Number is 1.0 (ideal), the lowest possible value. The condition number increases with increasing peak overlap.

8.12.4 Examine the data for all capillaries before accepting the spectral calibration results to be sure they each meet the following requirements:

- Quality Value minimum – 0.95
- Condition Number Maximum – 5.5
- Order of peaks in spectral profile from left to right should be:

  Blue → green → yellow → red

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- Peak morphology in the spectral profile – peaks are separate and distinct with no gross overlaps, dips, or irregularities
- Order of peaks in the raw data profile from left to right should be:

  Red → yellow → green → blue

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- No extraneous peaks in the raw data should appear.
- No excessive pull-up in the calibrated data (e.g., large yellow peak under red peak) should appear.

8.12.5 If any of the above criteria are not met, the calibration has failed.

8.12.5.1 The spectral calibration can be re-run. If problems persist, refer to the "Spectral Calibration Troubleshooting" section in the Applied Biosystems 3500xL Genetic Analyzer user guide.

8.13 A spectral calibration can run up to three injections. The number of injections performed depends on the number of capillaries that pass or fail during an injection and whether the "Allow Borrowing" option is selected. If borrowing is allowed, then the capillaries can share information with adjacent capillaries within an injection or between injections. Up to three borrowing events can be allowed.

8.14 Once all capillaries have been reviewed and accepted, click Accept.

8.15 The most recent spectral calibration is maintained in the software.

9 Long periods of inactivity

9.1 If the ABI 3500xL Genetic Analyzer remains unused for an extended period of time (approximately 2 weeks or longer), it is recommended that either a blank run (consisting of only formamide and size standard) be completed *or* the capillary array removed and the capillary ends stored in Type I H_2O until further use.

APPENDIX I – PREPARING NIST TRACEABLE SAMPLES

1 Take blood samples from at least 2 people, apply this material to blood stain cards (1 donor on each card) and dry the cards.

2 Demonstrate that the dried samples from each donor are homogenous by taking multiple cuttings from each stain card prepared and process the samples in accordance with the DNA extraction and typing procedures outlined in the Procedures Manual. All normal controls must be processed with samples (e.g., reagent blanks, negative blanks, positive controls, etc.). The same DNA profile should be obtained from the duplicate samples from each card.

3 Once the representative testing has been completed as outlined above take a single cutting from each stain card (e.g., if enough blood was drawn to make 2 stain cards from each donor for a total of 4 stain cards then a single cutting from each card is needed) and extract these samples in parallel with samples from a Standard Reference Material (SRM). If the correct DNA profiles are obtained from the in-house prepared samples and the SRM kit samples, then the in-house prepared samples are now considered National Institute of Standards and Technology (NIST) traceable.

4 To conduct testing using the in-house prepared NIST traceable samples take a single cutting from each stain card (e.g., if enough blood was drawn from an individual to make 2 stain cards a single sample from 1 of the cards is needed). Process the samples in accordance with the DNA extraction and typing procedures outlined in the Procedures Manual. Maintain all documentation in a properly labeled binder or file.

5 The in-house prepared samples are good until the sample is consumed or a discrepancy is detected. New lots of NIST traceable samples have to be prepared as listed in step 1 above (meaning you cannot just bleed the same people and use their sample without going through the representative testing with all the applicable extraction and typing controls).

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APPENDIX J – GUIDE FOR REVIEW OF AUTOSOMAL DNA DATA

Extraction worksheet
- Samples extracted from low to high (batch or per case)
- Knowns extracted separately from evidence
- Appropriate reagent blanks used and correct volumes
- Extraction method, volume, buffer and analyst specified

BIOMEK® worksheet
- Samples & controls in proper location
- Knowns separated from evidence
- "Wells Loaded" box accurately recorded
- Operator/Analyst actions initialed and dated
- Crosscheck initialed and dated or otherwise marked
- Instrument ID recorded, if applicable

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Plexor® worksheet
- Operator initials present
- Proper documentation if STD curves adjusted
- R^2 values for Autosomal/Y curves recorded
- IPC value checked for possible inhibition
- Samples noted that are not going to be amplified here or on the sample dilution tracking form, if applicable
- Instrument ID recorded, if applicable

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Sample Dilution Tracking worksheet
- Operator initials present
- Deviations correctly noted, if applicable

Reagent / Amplification worksheet
- Lot #s recorded
- Extraction / Quantitation / Amplification dates recorded
- TC #/Name (if applicable), unique positive and negative info recorded, date recorded

Manual Amplification form
- Manual dilutions checked
- Reagent blank volumes mimic the most sensitive samples they monitor

CE Plate map
- Reagent volumes & lot #s recorded, if applicable
- Sample load volumes & injection times recorded
- Plate name, instrument ID, plate loading date, and wells loaded recorded
- Controls evaluated and documented in box checked off and dated by examiner & tech reviewer
- Reagent blanks loaded and injected proper volume
- Minimum two ladders loaded per plate
- Reason for reinjection documented, if applicable

CE Data / Electronic Information
- Correct module file & peak threshold values used
- Verify ILS for each sample is suitable
- Verify ladder(s) allele values are correct
- Verify correct types obtained for at least one positive control associated with the amplification
- Negative control(s)/reagent blanks evaluated, proper action taken if expected values not obtained
    - Include a review of raw data to ensure primer peaks are observed indicating the sample was loaded
- Evaluate for off-scale data – use with caution if evidence or mixture sample
- Verify sample allele calls/edits/ST calculations are accurate, if applicable

Egram printouts

- Injection time recorded on egrams or in case file documentation
- ST, M/m calculation, if applicable, are correct

Landscape worksheet (if present)

- Verify allele calls against egrams
- INC loci addressed if not addressed elsewhere
- Profile suitability for comparison/searching documented, if not documented elsewhere

CODIS Specimen Detail report printouts

- All appropriate samples entered into CODIS
- Appropriate specimen nomenclature used
- Appropriate specimen category assigned
- Source ID appropriately chosen, if applicable
- Correct alleles entered
- CODIS review documented, if applicable & not documented elsewhere
- Reported NDIS suitability consistent with data entry

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CODIS search result printouts

- Staff index search conducted, if appropriate
- Local & state search sheets included, if appropriate
- Proper indices searched
- Matches properly evaluated & documented
- Appropriate alleles used

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Statistics

- Appropriate calculation used
- For LR, appropriate assumptions made
- For CPI, loci used with all data above stochastic threshold
- For single source, profile evaluated for 2p
- Theta used as appropriate
- ST peaks considered, if appropriate
- Correct alleles entered
- Correct database used
- Sample description / identification listed

APPENDIX K: GUIDE FOR REVIEW OF Y-STR DNA DATA

Reagent/Amplification worksheet
- Lot #s recorded
- Dilution/Amplification date recorded
- TC #/Name recorded

CE Data worksheet
- Lot #s recorded
- Proper controls

Electropherogram data
- Case number and item number documented
- Artifactual peaks addressed, if not done so in GeneMapper® ID-X
- Controls evaluated, proper action taken if expected values not obtained
- Appropriate parameters used

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Statistics
- Correct alleles entered
- STH applied, if applicable
- Sample description/identification documented

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