Procedure for Calibration and Equipment Maintenance

1.0 Purpose - This procedure specifies the required elements for the performance check, verification and maintenance of equipment used by the Forensic Biology Section as performed by the DNA Quality Control Officer or designee(s).

2.0 Scope – This procedure applies to equipment used by the Forensic Biology Section.

3.0 Definitions – See section Definition list

4.0 Equipment, Materials and Reagents

- NIST traceable digital thermometer
- Ice Shaver/crusher
- Purified dH_2O
- dH_2O
- NIST traceable weight set
- Spectral calibration kits (for ABI 7500, 3500 or equivalent)
- Microwave
- ~1000 mL beaker
- Syringe
- Septa
- 10x buffer
- Wipes (delicate task wipes)
- 50 mL conical tube
- 96 well reaction trays
- Pipettes
- Pipette tips
- Matrix standard set DS-33 to analyze automatically the five different colored fluorescent dye-labeled samples in a single capillary and PowerPlex™ 6C Matrix Standard and Matrix Dilution Buffer.

5.0 Procedure

5.1 ABI 7500 Real-Time PCR System Instrument Maintenance

5.1.1.1 The following table displays the recommended 7500 instrument and laptop maintenance schedule ensuring proper operation of the instrument (found in the AB 7500 Real-Time PCR Systems System Maintenance Guide (Part 4387777 Rev D 6/2010). Monthly, quarterly, semi-annual, and annual maintenance tasks should be performed using the listed steps/reference information at the frequencies indicated by the guide. All tasks except for annual maintenance shall be performed under the direction of the QCO and all records shall be maintained in the section. Monthly maintenance tasks are already included in months during which semi-annual or annual maintenance is performed.

Frequency	Maintenance Task	See Step:	Reference*
Monthly	check lamp status	---	116
	replace lamp if needed (NOTE: lamp replacement must be followed by ROI calibration/optical calibration/dye calibration—steps/references are noted in semi-annual task list of this maintenance table)	---	121-123
	decontaminate block	---	117-120
	background calibration	---	77-85, 88
	reboot laptop and wipe surface of 7500 with lint-free cloth	---	---
Quarterly	disk cleanup/defragment disks	---	---
Semi-annually (in order as listed)	check lamp status (replace if needed)	---	116
	decontaminate block	---	117-120
	ROI calibration**	---	65-75
	background calibration	---	77-85, 88
	optical calibration**	---	86-87
	dye calibration: VIC, FAM, ROX, NED (Quantifiler Duo)**	---	89-102
	Instrument verification (either RNase P or QC check)	5.2.3	103-112
	reboot laptop and wipe surface of 7500 with lint-free cloth, back-up of .eds files	---	---
Annually	Performed by AB technician; includes all semi-annual procedures	---	---
As needed	decontaminate block	---	117-120
	Laptop hard drive – run disc clean-up and defragmentation	---	64
	check lamp status	---	116
	replace lamp if needed (NOTE: lamp replacement must be followed by ROI calibration/optical calibration/dye calibration—steps/references are noted in semi-annual task list of this maintenance table)	---	121-123
	replace 7500 fuses	---	124-125
	RNase P verification (NOTE: RNase P verification is typically only performed at the request of AB Technical Support for troubleshooting purposes)	---	103-112
	Monitor the 7500 System	---	114-115
	update windows operating system	call AB	---
	update 7500 software	call AB	---

5.1.2 Repair: If an ABI 7500 becomes inoperable due to a need for repair by the manufacturer, the QCO shall immediately notify the DNA Technical Leader and manufacturer. Additionally, the QCO shall notify all members of the Forensic Biology Section via email and place a notice on the specific instrument that it is not available for use.

5.1.3 Post Annual Maintenance/Repair Performance QC Check: Before any validated ABI 7500 may be used for analysis following repair or annual preventive maintenance, a QC check shall be performed by the QCO. This QC check shall be performed as follows:

5.1.3.1 A NIST-TS (see DNA Reagent Preparation and Quality Control Procedure) shall be quantitated (i.e., Quantifiler Trio) with the DNA Standard Curve in duplicate (16 total data points) and a Negative Template Control (NTC).

5.1.3.2 Items listed above shall be quantitated in accordance with the Procedure for DNA Quantitation.

5.1.3.3 The NIST-TS shall indicate the presence of DNA. All testing negatives shall have an IPC C_t value of ≥ 40 or a value stating it is "Undetermined."

5.1.3.4 Quality metrics of the standard curves (Small autosomal, Large Autosomal, and TY) shall fall within acceptable QC ranges.

5.1.3.5 If either **5.1.3.3** or **5.1.3.4** is not satisfied, the QCO shall repeat the QC check.

5.1.3.6 The QCO shall notify the Section via email as well as by placing a notice on the specific instrument that it is again available for use once the QC check is completed.

5.1.3.7 The QCO shall document the testing performed and retain them in the appropriate QC files with the specific ABI 7500 maintenance records until it is scanned into the Section shared folder.

5.2 ABI 3500 Genetic Analyzer

5.2.1 Weekly Maintenance shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C.

5.2.1.1 Weekly Tasks:

5.2.1.1.1 Restart the computer and instrument.

5.2.1.1.2 Check storage conditions of the used arrays to ensure the array tip is covered in the reservoir.

5.2.1.1.3 Run the Wash Pump and Channels wizard.

5.2.1.1.4 Use the lab wipe to clean the anode buffer container valve pin assembly on the polymer delivery pump.

5.2.2 Spatial Calibration

5.2.2.1 Spatial calibration shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C.

5.2.2.2 Spacial Calibration shall be performed when:

5.2.2.2.1 The capillary array has been removed or replaced.

5.2.2.2.2 The detector door has been opened or the detection cell has been moved.

5.2.2.2.3 The instrument has been moved.

5.2.3 Spectral Calibration

5.2.3.1 Spectral calibration shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C.

5.2.3.2 Spacial Calibration shall be performed when:

5.2.3.2.1 The capillary array has been changed.

5.2.3.2.2 A service engineer has performed an optical service procedure such as realigning or replacing the laser or CCD camera or mirrors on the instrument.

5.2.3.2.3 A decrease in spectral separation (pull-up) in the raw or analyzed data has been observed.

5.2.3.3 Changing the Capillary Array - When a capillary has repeated ILS (i.e., sizing standard) failure, or the bases of the alleles in samples broaden, or the background noise in the electropherograms becomes repeated and excessive (based upon the training and experience of the Forensic Scientist), the array shall be replaced. Forensic Scientists shall notify the QCO and DNA Technical Leader if they observe any of the above-mentioned scenarios.

5.2.3.3.1 Changing the array shall be performed as per the "Applied Biosystems 3500/3500xL Genetic Analyzer User Guide; Part Number 4401661; Revision C."

5.2.3.3.2 Once completed, a spatial calibration shall be performed. A spectral calibration may be performed.

5.2.4 Service and/or Repair

5.2.4.1 Repair: If a 3500 becomes inoperable, the QCO shall notify the Section via email as well as by placing a notice on the specific instrument that is not available for use. The QCO shall also notify the DNA Technical Leader and the manufacturer that repair is needed.

5.2.4.2 Performance QC Check: If a 3500 instrument is removed from use due to repair, a post maintenance QC check on the instrument shall be performed by the QCO prior to its return to use in the Section.

5.2.4.3 Laser Failure/Replacement

5.2.4.3.1 If the Laser is replaced, a spatial and spectral calibration shall be performed.

5.2.4.3.2 The QCO shall then perform a Post Maintenance Performance QC Check on the instrument.

5.2.4.3.3 Additionally, a sensitivity study shall be performed on the instrument by the QCO at the direction of the DNA Technical Leader.

5.2.4.3.4 After all conditions are satisfied, the DNA Technical Leader shall release the instrument for use in Casework. The QCO shall notify the Section by email and by placing a notice on the specific instrument that it is available for use.

5.2.5 Annual Preventative Maintenance

5.2.5.1 The ABI 3500 Genetic Analyzers shall have preventative maintenance performed annually by the manufacturer for instruments in use by the section for the entire year.

5.2.5.2 Performance QC Check: After preventative maintenance, each 3500 shall have a post maintenance QC check performed by the QCO.

5.2.6 Documentation of any repair or annual preventative maintenance, as well as subsequent QC Checks, shall be retained in the Section indefinitely and shall be maintained by the QCO in the binder associated with each specific instrument which shall be located near that specific instrument.

5.2.7 **Post Maintenance Performance QC Check:** Before any validated 3500 shall be used by Forensic Scientists in the Forensic Biology Section after repair or maintenance, a Performance QC check shall be performed by the QCO as described below. When a 3500 instrument is either removed or returned to service, the QCO shall notify the Section via email and place a notice on the instrument regarding its status. All QC documentation shall be retained in the appropriate QC files with the specific 3500 maintenance records. DNA TL approval shall be obtained prior to return to service of any 3500.

5.2.7.1 **Post Annual Preventative Maintenance:** If no modifications to the optical components of a 3500 are made during annual preventative maintenance (PM), the following instrument assessments shall be performed:

5.2.7.1.1 Precision: A master mix of ladder/formamide/WEN sizing standard shall be prepared to fill a complete injection (24 wells or 3 columns) on a single plate and injected at normalized conditions. The ladder shall be analyzed for precision such that all alleles within the allelic ladder have standard deviations below 0.15 bp.

5.2.7.1.2 NIST Traceability: After precision (and normalization, if required) has been successfully achieved, at least one NIST-Traceable Standard (NIST-TS) (see Procedure for DNA Reagent Preparation and Quality Control) must be amplified with the appropriate amplification positive and negative control(s) and injected on the serviced instrument. The NIST-TS, positive amplification control and allelic ladder must provide the expected allele calls at all loci tested. All negative controls must be free of alleles. Note: If no modifications

were made to the optical components of the 3500, only NIST Traceability shall be performed.

5.2.7.1.3 Note: If any of the above assessments are not satisfied, the QCO may repeat the assessment one additional time. If the assessment fails a second time, **proceed to 5.2.7.2** or place a service call to the manufacturer as necessary.

5.2.7.2 **After Repair/Post PM QC Failure:** After any repair to the optics system of a 3500 (e.g., laser replacement), or if post annual maintenance QC fails, the following assessments shall be performed:

5.2.7.2.1 Precision and NIST-TS (see **5.2.7.1.1** and **5.2.7.1.2**).

5.2.7.2.2 Sensitivity: A dilution series of NIST-TS shall be prepared beginning with a total initial input target of 2 ng and serially diluted to 0.031 ng, with an additional 0.100 ng dilution prepared. The serial dilutions shall be quantified in triplicate and their concentrations adjusted accordingly (if necessary). The dilutions shall then be amplified in triplicate and injected on the 3500 at previously established conditions. All data within 60 to 500 bp shall be assessed for minimum threshold (2[max-min], Limit of Detection, Limit of Quantification), peak height imbalance (peak height ratios), and stochastic thresholds (allelic drop-out). Thresholds and PHR shall fall within existing specifications. Note: Other well-characterized DNA samples may be used as appropriate for this assessment.

5.2.8 Documentation of any repair or annual preventative maintenance, as well as subsequent QC Checks, shall be retained in the Section indefinitely and shall be maintained by the QCO in the binder associated with each specific instrument which shall be located near that specific instrument.

5.3 ABI 9700 Thermal Cyclers

5.3.1 **Internal Quarterly Verification:** All thermal cyclers currently in service within the Section shall be subjected to a series of temperature verifications on an annual basis by the QCO. Gloves, masks and lab coats shall be worn at all times. Caution shall be exercised at all times as the thermal cyclers can reach temperatures in excess of 100 °C. Documentation of all verifications shall be noted on the Thermal Cycler Verification Record by the QCO performing the verification for each thermal cycler. This documentation shall be retained indefinitely by the QCO.

5.3.1.1 **Temperature Uniformity:** A set of twelve wells on each thermal cycler shall be tested for two temperature groups: 95 °C and 40 °C. For each temperature group, the range between the highest and lowest values shall not exceed +/- 1 °C. Additionally, each individual well, for each individual temperature, shall not deviate +/- 1 °C from the set temperature.

5.3.1.1.1 Turn on the thermal cycler, select "run" and "TNU" (or "Temp Uniformity"). Select a 25 µL reaction volume. Select "start."

5.3.1.1.2 When the thermal cycler reaches 95 °C as indicated on the display, select the "pause" button. Insert the digital Eutechnics 4500 probe (or equivalent) into the appropriate well (listed below) and shut the lid. Do not pinch the cord. Tested wells are as follows: A1, A6, A12, C4, C9, D7, E3, F2, F11, H1, H7, and H12.

5.3.1.1.3 Allow the probe to stabilize (may take a few minutes). Record the temperature to the nearest tenth of a degree for that well as indicated by the probe. Proceed to the next well until all twelve wells have been recorded on the Thermal Cycler Verification Record (TCVR) for the 95 °C temperature range. Note: QCO or designee may have to continue selecting the "pause" mode to complete this step so as to keep the thermal cycler at 95 °C.

5.3.1.1.4 Select the "resume" button on the display or allow the "pause" mode to time out. The thermal cycler begins to cool down to 40 °C. Using the same wells as listed in **5.3.1.1.2**, test and record the 40 °C temperature results as described in **5.3.1.1.2** and **5.3.1.1.3**. Record the temperature for each well on the TCVR.

5.3.1.1.5 Calculate the range in temperature from **5.3.1.1.3** to **5.3.1.1.4**. These values shall not exceed +/-1 °C from the set temperature or from each other (compare highest recorded temperature for each range to the lowest recorded temperature for each range). Record the results on the TCVR.

5.3.1.1.6 If the calculated values exceed the criteria described in **5.3.1.1**, the QCO shall notify the DNA Technical Leader and the thermal cycler in question shall be removed from service. The QCO shall notify the Section via email, as well as by placing a "Do Not Use" sticker on the thermal cycler.

5.3.1.2 **Heat and Cool Rate Test:** The ability of the thermal cycler to heat and cool the block quickly is determined by the following steps:

5.3.1.2.1 Turn on the thermal cycler, select "Utilities," "Diag," "System," and "Rate."

5.3.1.2.2 The thermal cycler displays a warning. At this time, place an empty 3130XL 96- well tray, with septa, onto the thermal cycler, and close the lid. Select "Cont."

5.3.1.2.3 The thermal cycler runs the program. When the program is completed, the display indicates "pass" or "fail." It also provides the rate at which the thermal cycler both heats and cools. Rates, as well as "pass" or "fail," shall be recorded on the TCVR.

5.3.1.2.4 If a "fail" result is obtained, the QCO may retest the thermal cycler once more. If the particular thermal cycler indicates a second "fail," the QCO shall notify the DNA Technical Leader and the thermal cycler in question shall be

removed from service. The QCO shall notify the Section via email, as well as by placing a "Do Not Use" sticker on the thermal cycler.

5.3.1.3 **Temperature Verification:** The digital probe is used to verify that the thermal cycler is producing a temperature within +/- 1 °C of the set temperature.

5.3.1.3.1 In the display window, select "Utilities," "Diag," "TempVer."

5.3.1.3.2 Place the digital probe into well A6 and close the lid. Do not pinch the cord. Select "Run."

5.3.1.3.3 The thermal cycler ramps up to 85 °C and prompt the user when complete. At this point, the QCO records the temperature on the digital probe on the TVCR and enter that value as prompted into the thermal cycler to the nearest tenth of a degree.

5.3.1.3.4 Continue the test by allowing the thermal cycler to ramp down to 45 °C ("stabilizing at setpoint") and record the resulting information as described in

5.3.1.3.3. Select "accept" once both the 85 °C and 45 °C values are entered into the display window.

5.3.2 Notification for Use: If any thermal cycler fails any of the three tests, the QCO shall immediately notify the DNA Technical Leader, as well as the Section via email and a "Do Not Use" sticker shall be placed on the affected instrument.

5.3.3 Performance QC Check: If a thermal cycler requires a QC check after repair, for validation, or before a new instrument is put on-line, a QC check shall be performed by the QCO, in addition to the three tests as described in **5.3.1**.

5.3.3.1 This QC check shall consist of the amplification of the following as a set:

5.3.3.1.1 Positive amplification control (2800M) and negative amplification control (Neg Amp), using the current amplification kit.

5.3.3.1.2 NIST-TS.

5.3.3.2 Five total sets shall be amplified at the following well locations and electrophoresed and analyzed per DNA procedures:

5.3.3.2.1 E1-H1, C4-F4, B7-E7, E10-H10, A12-D12

5.3.3.3 The expected results for the NIST-TS, positive amplification controls, and allelic ladders shall be obtained for all loci and the alleles shall be balanced within and between loci and peak heights above the analytical threshold and < 15000 RFUs. All negative controls shall be free of any peaks or activity. If any of these conditions are not met (for reasons other than instrument failure, known artifacts), then the QCO may retest the affected

wells in the thermal cyclers once. If the conditions are not met this second time, the QCO shall keep the thermal cycler offline and notify the DNA Technical Leader and manufacturer. If the thermal cycler is under a manufacturer warranty, the manufacturer shall be contacted for repair. If the thermal cycler is no longer under any warranty, it shall be placed in storage for eventual surplus.

5.3.4 External Calibrations/Verification: If the thermal cyclers are verified by an external vendor, the results shall be documented. The thermal cyclers that are passed by the external vendor shall be accepted as calibrated/verified and noted as such until the next quarterly verification is due. This documentation shall be retained indefinitely by the QCO.

5.4 Digital Probes

5.4.1 Annual External Calibration: the digital probes (Eutechnics 4500 or equivalent) shall be calibrated annually by a contract vendor against an appropriate NIST traceable standard.

5.5 Bulb Thermometers

5.5.1 Purpose/Use: Used to measure temperatures in incubators. Surplus calibrated bulb thermometers shall be retained by the QCO, unless broken and then they shall be disposed of in accordance with the Procedure for Section Safety.

5.5.2 Annual Internal Performance Check: All bulb thermometers in use within the Forensic Biology Section shall be checked on an annual basis internally against a NIST traceable thermometer (i.e., the "NIST lollipop") in an ice bath.

5.5.2.1 Freeze several trays of dH_2O into ice cubes; once frozen, grind or crush them in an ice shaver (or equivalent). Mix the ice shavings with dH_2O and place into an insulated container deep enough (thermos or equivalent) to contain the metal probe portion of the NIST Traceable Thermometer.

5.5.2.2 The QCO shall wipe down each bulb thermometer with fresh 10 % bleach followed by an ethanol rinse and allow it to dry (either through evaporation or wiping with a wipe) before inserting it into the ice bath.

5.5.2.3 Using clamps and foam (or equivalent) to hold both the NIST traceable thermometer and the bulb thermometer to be calibrated within an inch of each other in the ice bath, wait for the NIST traceable thermometer to register 0.0 °C. Be sure to align the bulb thermometer such that the bulb portion is submerged in the ice bath, but that the area marked for 0.0 °C can be visualized by the QCO.

5.5.2.4 Once the NIST traceable thermometer reads 0.0 °C, record the temperature to the nearest tenth of a degree on the bulb thermometer. If the bulb thermometer is greater than +/- 1 °C from the NIST traceable thermometer, it shall be destroyed and replaced with a calibrated bulb thermometer.

5.5.2.5 The QCO shall record both the NIST traceable thermometer and calibrated bulb

thermometer readings on the Bulb Thermometer Temperature Performance Check Form. The QCO shall also create and place a sticker on each calibrated bulb thermometer that indicates the specific bulb thermometer number, the date the next performance check is due, the initials of the QCO performing the check, and whether the user of the bulb thermometer shall add or subtract tenths of a degree to the reading of that bulb thermometer to bring it to specifications as indicated by the NIST traceable thermometer (i.e., if the bulb thermometer reads 0.5 °C higher than the NIST traceable thermometer, the Forensic Scientist shall subtract 0.5 °C from the bulb thermometer reading before recording a temperature).

5.5.2.6 This process shall be completed for all bulb thermometers, including those set aside for storage or future use (i.e., replacement).

5.5.2.7 Documentation of the performance checks shall be retained indefinitely by the QCO in the Section.

5.6 Digital Thermometers: Purchased from external vendor; shall be NIST traceable and replaced when NIST traceability expires. Digital thermometers shall be used to monitor temperatures on freezers and refrigerators in the Section as needed. Surplus digital thermometers shall be retained by the QCO.

5.7 NIST Traceable Thermometer (i.e., the "NIST lollipop"): Has an elongated metal probe which is used for testing against bulb thermometers purchased from an external vendor; shall be NIST traceable and replaced when NIST traceability expires.

5.8 Balances - External Calibrations: All balances in the Forensic Biology Section shall be calibrated at least annually by a contract vendor. The weight set in the Forensic Biology Section shall be calibrated annually.

5.9 Pipettors

5.9.1 Annual External Calibrations: All pipettors in the Forensic Biology Section shall be calibrated at least annually by a contract vendor.

5.9.2 Repair: If a pipettor breaks or a Forensic Scientist based on their training and experience believes that the pipettor does not work properly, it shall be given to the QCO for storage until an external calibration vendor can repair and calibrate it. If the pipettor is not repairable, it shall be removed from the Section.

5.10 Temperature Data Loggers

5.10.1 Used to monitor temperature in post amplification rooms where 3130XLs (or equivalent) are currently in use.

5.10.2 Data Loggers (USB)

5.10.2.1 Annual External Calibrations: All data loggers in the Forensic Biology Section shall be calibrated annually by a contract vendor.

5.10.2.2 Retention of Data: The data loggers shall be set to record data every five minutes. The

data shall be printed by the QCO every month (to coincide with monthly 3130XL maintenance) and retained indefinitely in the Section by the QCO. The data shall include the date range captured by the logger as well as the serial number of the logger. Once monthly data is captured and retained, the data logger shall be cleared to record data for the next month.

5.10.3 Retention of data: The paper temperature discs shall be changed weekly when in use by the QCO. The circular discs shall be scanned into digital images. Both the original disc and the digital image shall be retained indefinitely by the QCO in the Section.

5.11 Centrifuges

5.11.1 Annual Preventative Maintenance: The Beckman-Coulter Allegra X-12R and X-12 centrifuges shall have annual preventative maintenance performed by the manufacturer. The manufacturer shall place a maintenance sticker on the centrifuge documenting that the service was performed.

5.11.2 Repair: If repairs are necessary, the manufacturer shall be notified by the QCO and an "out of use" sticker placed on the affected centrifuge notifying the Section of its unavailability. Once the affected centrifuge is repaired, the QCO shall remove the "out of use" sticker.

5.12 Biosafety Cabinets/Chemical Fume Hoods/Laminar Flow Clean Air Benches

5.12.1 Annual External Calibrations: All Nuaire Biological Safety Cabinets, Chemical Fume Hoods, and Laminar Flow Clean Air Benches (amplification hoods) in the Section shall be calibrated annually by a contract vendor.

5.12.2 Any hood listed in **5.12.1** that does not pass certification shall not be used.

5.13 Freezers/Refrigerators

5.13.1 Recording Temperatures: The QCO shall record high and low temperatures for all common area refrigerators/freezers in the Section weekly; however, if the QCO has not yet recorded the temperature and a Forensic Scientist uses a common area refrigerator/freezer, the Forensic Scientist shall record the temperatures prior to opening the door(s). Temperatures are recorded on a Temperature Recording Form (TRF).

5.13.2 -20 °C Freezers: These freezers shall not vary more than + 5 °C from the set temperature. The temperature for these freezers shall be recorded by personnel using the TRF as described in **5.13.1**.

5.13.2.1 The QCO shall fill out all required information regarding freezer serial number, the location of the freezer, the set temperature of the freezer, and the associated digital thermometer serial number at the beginning of every calendar year on a TRF for each common area -20 °C freezer.

5.13.2.2 If at any point during the calendar year a new digital thermometer is needed, the QCO shall write at the bottom of the TRF the date on which a new thermometer was used and the serial number for the new thermometer.

5.13.2.3 If a -20 °C freezer must be thawed, the contents of the freezer shall immediately be moved to another -20 °C freezer that is within range and the QCO shall note this, as well as the affected dates, on the TRF. The contents shall not be returned to the original -20 °C until the temperature is within range.

5.13.2.4 If the temperature for a -20 °C freezer exceeds the + 5 °C consistently for more than 5 consecutive business days, QCO shall immediately move the contents to another -20 °C freezer that is within range and note this, as well as the affected dates, on the TRF. The contents shall not be returned to the original -20 °C freezer until the temperature is within range.

5.13.3 -10 °C/4 °C Freezer/Refrigerator Units: These units shall not vary more than + 5 °C from the set temperature(s) for the freezer portion; the refrigerator portion shall not fall below 0 °C or exceed 9 °C. The temperature for these freezers shall be recorded using the TRF by personnel as described in **5.13.1**.

5.13.3.1 The QCO shall fill out all required information for common area -10 °C/4 °C freezer/refrigerator units regarding the unit serial number, location, set temperatures, and the associated digital thermometer serial number on the TRF. For limited access -10 °C/4 °C freezer/refrigerator units, the Forensic Scientist(s) that have access to such units shall fill out the information on a TRF.

5.13.3.2 If at any point during the calendar year a new digital thermometer is needed, the QCO shall be notified and the new thermometer serial number shall be recorded on the TRF associated with the refrigerator/freezer.

5.13.3.3 If the QCO or Forensic Scientist observes temperatures out of the range specified in **5.13.3** for more than five consecutive business days, then the QCO (for common area units) or the Forensic Scientist (limited access units), shall attempt to adjust the temperature back in range using the thermostat for the unit. If the temperature does not come within range within an 24 hour period, the QCO (or Forensic Scientist) shall transfer the contents of the unit to another unit with the same temperature parameters and note on the TRF the unit to which the contents were transferred and the date of transfer. If additional adjustments of the thermostat are unsuccessful, the unit shall be removed from service and clearly marked as being out of service. If additional adjustments are successful at restoring the unit to the temperatures specified in **5.13.3**, then the contents may be returned to the unit.

5.13.4 -70 °C Baxter Scientific Cryo-Fridge/-39 °C Revco Cryo-Fridge: These units shall not vary more than + 10 °C from the set temperatures. The temperatures for the freezers shall be recorded using the TRF by personnel as described in **5.13.1**.

5.13.4.1 The QCO shall fill out all required information regarding freezer serial number, the location of the freezer, the set temperature of the freezer, and the associated digital thermometer serial number at the beginning of every calendar year on a TRF for each freezer.

5.13.4.2 If any cryo-fridge deviates consistently + 10 °C from the set temperature for more than 5 consecutive business days, the QCO shall be notified, the contents of the affected cryo-fridge removed and stored in an equivalent location, and the cryo-fridge manufacturer (or current contract vendor) notified for repair. All information regarding cessation of use and relocation of contents shall be documented on the TRF.

5.14 **Incubators**: Temperatures shall be recorded on the day(s) the incubator is in use. If the incubator is in a common area, the QCO shall record the temperature. If the incubator is in a shared suite, the Forensic Scientist shall record the temperature. Temperatures shall be recorded on a TRF specific for the incubator.

5.14.1 The QCO or Forensic Scientist shall fill out all required information regarding the unit serial number, location, set temperatures, and the associated bulb thermometer number on the TRF.

5.14.2 If the incubator is not used, the QCO or Forensic Scientist shall strike through the box which corresponds to the day(s) not in use.

5.14.3 The incubators shall be +/- 5 °C degrees within the set temperature. If an incubator consistently deviates more than this over a period of five consecutive readings, then the QCO or Forensic Scientist shall attempt to adjust the temperature back into the acceptable range over a period of 24 hours. If all attempts at obtaining a set temperature within range fail, the QCO shall be notified and the incubator removed from service and marked as such.

5.15 All verification, calibration, maintenance, and QC documentation shall be retained in the Forensic Biology Section.

5.16 When any of the following instruments/equipment need repair and are taken out of use from the Section, the QCO shall notify the DNA Technical Leader, and if necessary, the manufacturer. The QCO shall also notify the DNA Technical Leader when the instruments/equipment are suitable for use by the Section.

- 3130XL, ABI 7500, ABI 9700, QIAgility, EZ1 Advanced XL BioRobot, Centrifuges, Hoods, Freezers/Refrigerators, Balances.

5.17 QIAgility

5.17.1 Annual Preventative Maintenance

5.17.1.1 Annual Preventative Maintenance: the QIAgility shall have preventative maintenance performed annually by the manufacturer.

5.17.1.1.1 Refer to the Planned Maintenance Protocol (record) provided by the manufacturer for specific calibrations, verifications, and tests performed during the annual preventative maintenance.

5.17.1.1.2 Performance QC Check: After preventative maintenance, each QIAgility shall have a post maintenance QC check performed by the QCO.

5.17.2 Weekly Maintenance

5.17.2.1 Wipe the outside of the instrument with a dust cloth or lab wipe dampened with deionized water.

5.17.2.2 Remove all loading blocks and the tip ejector chute from the worktable. Rinse these with ethanol and dry.

5.17.2.3 Wipe the worktable down with ethanol and return the tip ejector chute to the worktable. Note: The tip ejector must be re-calibrated each time it is removed and replaced.

5.17.2.3.1 In the menu bar, click Options and then click Setup tip ejector. Click "Yes."

5.17.2.3.2 Follow the prompts to calibrate the ejector. Click "Locate Ejector" then click "Yes."

5.17.2.3.3 A box will appear asking "Is the pipettor hub above the slot of the ejector?" Verify that the hub is located correctly and click "Yes."

5.17.2.3.4 Click Finished and then click "OK."

5.17.2.4 Close the lid and turn on the UV lamp. Click the light bulb on the icon bar at the top of the screen. Click the box marked "Close software when UV finished?" and set the time to 15 minutes.

5.17.2.5 Click Start and then click "Yes" when the alert screen opens.

5.17.2.6 Document the maintenance on the Forensic Biology Section QIAgility Maintenance Form (located with the instrument).

5.17.2.6.1 The QCO shall retain such information in the QC files with the specific instrument maintenance records.

5.17.3 Post Maintenance Performance QC Check: Before any validated QIAgility may be used by a Forensic Scientist after repair or maintenance, a Performance QC check shall be performed as follows:

5.17.3.1 A NIST-Traceable Standard (NIST-TS) and associated Neg K (see Procedure for DNA Reagent Preparation and Quality Control) shall be quantified/amplified/setup for capillary electrophoresis and run to the extent which the QIAgility has been validated.

5.17.3.2 Items listed in **5.17.2.1** shall be electrophoresed on the 3130XL at the normalized injection protocol.

5.17.3.3 For quant setup, the NIST-TS shall indicate the presence of DNA. All negative controls

(e.g., NIST, Neg K, NTC) shall have an IPC C_t value of ≥40 or a value stating it is "Undetermined." Quality metrics of the standard curves (both human and male) shall fall within acceptable QC ranges.

5.17.3.4 The NIST-TS, positive amplification control(s) and allelic ladder shall provide the expected allele calls at all the loci tested.

5.17.3.5 All negative controls (Neg K, amplification negative control(s)) shall be free of any alleles.

5.17.3.6 If **5.17.2.3, 5.17.2.4,** or **5.17.2.5** are not satisfied (for reasons other than instrument failure, known artifacts), the QCO or designee may retest (re-electrophorese, re- amplify, re-quantitate, or re-extract) the samples one more time. If the retest does not pass, a service call for equipment maintenance shall be placed.

5.17.3.7 If the instrument passes the QC check, the QCO shall notify the Section via email and place a notice on the specific instrument that it is available for use.

5.17.3.8 The QCO shall document the testing performed and retain such information in the QC files with the specific maintenance records.

5.18 EZ1 Advanced XL BioRobot

5.18.1 Annual Preventative Maintenance

5.18.1.1 Annual Preventative Maintenance: the EZ1Advanced XL BioRobots shall have preventative maintenance performed annually by the manufacturer.

5.18.1.1.1 Refer to the Planned Maintenance Protocol (record) provided by the manufacturer for specific calibrations, verifications, and tests performed during the annual preventative maintenance

5.18.1.1.2 Performance QC Check: After preventative maintenance, each EZ1 shall have a post maintenance QC check performed by the QCO.

5.18.2 Cleaning/Maintenance – See the Section Procedure for DNA Extraction Using the EZ1 Advanced XL.

5.18.3 Post Maintenance Performance QC Check: Before any validated EZ1 may be used by a Forensic Scientist after repair or maintenance, a Performance QC check shall be performed as follows:

5.18.3.1 A NIST-TS and associated Neg K (see Procedure for DNA Reagent Preparation and Quality Control) shall be extracted.

5.18.3.2 Items listed in **5.18.1.1** shall be quantified, amplified, and electrophoresed on the 3130XL following current Section procedures.

5.18.3.3 For quant setup, the NIST-TS shall indicate the presence of DNA. All negative controls

(e.g., NIST, Neg K, NTC) shall have an IPC C_t value of $\geq$ 36 or a value stating it is "Undetermined." Quality metrics of the standard curves (both human and male) shall fall within acceptable QC ranges.

5.18.3.4 The NIST-TS, positive amplification control(s), and allelic ladder shall provide the expected allele calls at all the loci tested.

5.18.3.5 All negative controls (Neg K, amplification negative control(s)) shall be free of any alleles.

5.18.3.6 If either **5.18.1.3, 5.18.1.4,** or **5.18.1.5** are not satisfied (for reasons other than instrument failure, known artifacts), the QCO or designee may retest (re- electrophorese, re-amplify, or re-quantify) the samples one more time. If the retest does not pass, then a service call for equipment maintenance shall be placed.

5.18.3.7 If the instrument passes the QC check, the QCO shall notify the Section via email and place a notice on the specific instrument that it is available for use.

5.18.3.8 The QCO shall document the testing performed and retain such information in the QC files with the specific maintenance records.

6.0 Limitations - As noted in **5.0**.

7.0 Safety

7.1 Thermal cyclers can exceed temperatures of 100 °C; use with caution to avoid burns.

7.2 Gloves, masks, and lab coats shall be worn when performing any verifications, calibrations, or QC checks described in Section 5.

7.3 If the ice shaver (or equivalent) used as described in **5.5** is not self-contained, safety glasses shall be worn during operation.

7.4 Formamide is a known chemical hazard; it causes eye, skin and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Wear appropriate eyewear, masks, gloves and clothing when in use.

8.0 References

Forensic Biology Section Procedure for Safety

Forensic Biology Section Procedure for Use of the3500 Genetic Analyzer for Casework

Forensic Biology Section Procedure for DNA Reagent Preparation and Quality Forensic

Biology Section Procedure for DNA Quantitation with Quantifiler® Trio

Forensic Biology Section Procedure for PCR Amplification using PowerPlex® Fusion 6C for Casework

Forensic Biology Section Procedure for PCR Amplification using PowerPlex® Y23

Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

Forensic Biology Section Procedure for DNA Extraction using the EZ1 Advanced XL

Instrument manuals

Applied Biosystems 3500 Genetic Analyzers. User Bulletin. 2005 Applied Biosystems. Part Number 4363787. Rev A. (or most recent revision)

Eutechnics 4500 Manual NIST

Special Publication 819

Applied Biosystems 7500/7500 Fast Real Time PCR Systems. Maintenance Guide. Part Number 4387777 Rev. D

9.0 Records

- Temperature logs for freezers, refrigerators (Daily and Weekly)

- Thermal Cycler Temperature Performance Check Forms
- Bulb Thermometer Calibration Forms
- Biosafety Cabinets/Chemical Fume Hoods/Lamina Flow Clean Air Benches Certificates
- Certificates of Calibration for NIST Traceable Digital Thermometer, Digital Thermometers, Balances, Pipettors, Digital Probes, Weights, and Temperature Chart Recorders/Data Loggers
- Manufacturer documentation of preventative maintenance and/or repair for 3500, ABI 7500s, EZ1s, QIAgilities, centrifuges
- EZ1 Cleaning/Maintenance Form
- QIAgility Maintenance Form
- 3500 Monthly Maintenance Form

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document
10/26/2012	2	Changed wording in 5.1.6.4 to remove requirement to note activity. Added notification requirement to 5.2.1.

12/22/2013	3	Replaced Section 5.2 7000 Instrument maintenance with new 7500 maintenance; Removed 7000 references, added 7500 Maintenance guide as reference; Changed 7000 to 7500 in definitions, equipment, 5.19, and records; 5.2.3 – removed requirement to place sticker on instrument; Added 7500Maintenance Guide as Attachment A; Removed diagram references
05/10/2013	4	5.2.1 - added wording to allow semi-annual/annual maintenance to cover monthly maintenance; 5.3.3.1.1 – removed reference to Identifiler kit; 5.3.3.2 – removed wording regarding injection parameters (refer to section procedures)
09/25/2013	5	Header – added issuing authority titles; 3.0 – added EZ1 and QIAgility to critical equipment list; 5.1.6 – changed performance check requirement; 5.16.3.2 – changed wording to correspond with section 5.16.1; removed DNA Database analyst throughout document; 5.19 – added EZ1 and QIAgility to list; 5.20 – inserted QIAgility maintenance; 5.21 – inserted EZ1 maintenance; 8.0 – added EZ1 extraction procedure
12/18/2013	6	2.0, 5.1.5.3.5, 5.1.6 – removed database unit reference, changed casework to Section; 3.0 – removed bead sterilizers and BioRobot;5.1.6.1.2 added NIST-TS; 5.8, 5.14 – removed section; 5.12, 5.16, 5.16, 5.18, 5.21 – updated section reference; 8.0 – updated procedure references
03/07/2014	7	5.18.1.6 – added form to record maintenance; 5.19.1 – added cleaning to section title; 9.0 - added EZ1 and QIAgility cleaning/maintenance forms to records
04/18/2014	8	3.0 – removed heat blocks; 5.1.2.19.4 – added notation for buffer lot; 5.1.2.28, 5.1.4.2.4 – updated tracking to form from FA; 5.1.3.1 – clarified when wash would be performed; 5.1.4.1.34, 5.1.4.2.3 – added spectral calibration requirement; 5.1.6.1.2 – added note clarifying when to perform; 5.1.6.1.3 – updated kit name; 5.1.8.2 – added requirement to be done for array change also; 5.1.8.5 – updated formamide amount and clarified note; 5.5.1 – updated use; 5.7 – removed bead sterilizers; 5.8.1.4 – removed "unless"; 5.8.1.5 – updated wording; 5.8.2 – added weight calibrations; 5.10 – added Data loggers and recording requirements; 5.13 – removed heat blocks section; 5.18.2.1 – clarified extent of QC check; 9.0 – updated records
08/29/2014	9	5.1.4.1.33 – changed record information on form, not in FA; 5.1.4.1.34, 5.1.4.2.3 – changes to spectral may be performed; 5.1.6.1.3 – added note for post maintenance check; 5.1.6.1.3, 5.2.3.1, 5.2.3.3, 5.3.3.1.2, 5.3.3.3 – removed requirement for Negative extraction control testing; 5.1.8.2 – removed requirement for spectral if array changed; 5.17.1, 5.18.1 – added annual preventative maintenance

02/27/2015	10	5.13.1, 5.13.3.2 – removed individual refrigerators, 5.13.1 - updated for weekly recording of high and low temperatures
12/28/2015	11	3.0 – updated name; 5.1.3.1 – added transferring of data to monthly maintenance for 3130; 5.2.1 – removed archive data from 7500; 5.2.3.7 – added wording about scanning records; 5.8.2and 5.9.1 – reworded to at least annually; 5.10.2 and 5.10.4 – removed temp chart recorders
12/20/2016	12	3.0 – moved to section list; 4.0 – updated to add 3500; 5.1 – removed ABI 3130, added AB 3500 to procedure in section 5.2; 5.4 – updated when verifications performed; 5.4.3 – updated wording for 3500 data; 5.17.3 – updated for Quant Trio; 8.0 – updated names, 10.0 – removed attachment (controlled document)