

Forensic Biology General Lab Maintenance Manual

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Section 1 Chemicals and Reagents

1.1 Introduction

By definition, "critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary or casework reference samples" (FBI QAS, 2009). Reagents which are used in pre-amplification procedures directly involved in DNA extraction from forensic casework or database samples, have been deemed critical reagents to prevent unnecessary loss of sample.

All post-amplification DNA reagents are hereby listed as non-critical reagents. Non-critical DNA reagents need not be verified prior to use in casework.

When a reagent fails to meet the criteria for verification, the DNA Technical Manager shall be notified and an appropriate course of action will be determined. The reagent shall not be used in casework unless or until the issue has been resolved and the approval or an alternate course of action suggested by the DNA Technical Manager has been documented.

1.2 General Instructions

- Chemical and reagent quantities may be adjusted to prepare more or less than the specified amount.
- All critical reagents prepared in-house shall be stored in sterile/autoclaved containers.
- Reagent containers are to be labeled with the following:
 - Name of reagent
 - Lot number (the date of preparation and preparer's 2 or 3 letter initials are used as the lot # for reagents prepared in-house and reagents where a lot # is not provided by the commercial vendor; i.e. 06-0101MLC would be the lot # for a reagent prepared on Jan. 1, 2006 by MLC)
- One member of the DNA discipline shall be designated for purchasing of supplies and reagents.
- All chemicals and reagents prepared or purchased shall be logged in the reagent log maintained in the DNA laboratory.
- All purchased chemicals/reagents are assigned the expiration date specified by the manufacturer. If no manufacturer expiration date is provided, the following guidelines apply:
 - Chemicals used in the in-house preparation of a reagent are not assigned an expiration date. Expiration dates are assigned to the prepared reagents as specified below.
 - Reagents used as received will expire one year from the date of receipt.

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- All newly received/prepared critical reagents and chemicals shall be verified prior to use on casework/database samples. Chemicals/reagents requiring verification should be clearly marked as such.

1.3 Chemicals and Reagents not Requiring In-House Preparation and/or Verification

Chemicals/Reagents purchased from a commercial vendor and requiring no preparation or verification prior to use in procedures or preparation of other reagents are listed below. They shall be stored as prescribed by the manufacturer and shall expire on the date provided by the manufacturer. Expiration dates are assigned as previously described, if not provided by the manufacturer and unless stated otherwise.

- α -Naphthyl Phosphate [solid]
- Aluminum Sulfate [solid]
- Anode Buffer Container, 3500 series from Life Technologies [liquid]
- Cathode Buffer Container, 3500 series from Life Technologies [liquid]
- Conditioning Reagent, 3500 series from Life Technologies [liquid]
- Dithiothreitol [solid]
- EDTA [solid]
- Ethanol, anhydrous reagent grade [liquid]
- Fast Blue B (o-Dianisidine Tetrazotized) [solid]
- Glacial Acetic Acid [liquid]
- Concentrated Hydrochloric Acid (HCl) [liquid]
- 3% Hydrogen Peroxide [liquid]
- Indigo Carmine dye [solid]
- Nuclear Fast Red [solid]
- Phenolphthalein [solid]
- PowerPlex[®] 16 Matrix Standards from Promega [liquid]
- POP-4 Polymer from Life Technologies [liquid]
- Potassium Hydroxide [solid]
- Saturated Picric Acid [liquid]
- Semen Standard [liquid]
- Sodium Acetate, anhydrous [solid]
- Sodium acetate buffer solution (3M, pH 5.2) [liquid]
- Sodium Hydroxide Solution (NaOH) [liquid]
- Sterikon[®] plus Bioindicator [ampules]
- Tris base [solid]
- Xylene substitute Substitute [liquid]
- Zinc [solid]

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1.4 Preparation and Verification

- Vendor supplied standard samples / positive control samples that are sent with PCR amplification kits may be discontinued or substituted at vendors' discretion. Scientist's bench notes and batch central log information will indicate the identity of positive control samples used in analysis.
- Similarly, variations in vendor supplied materials (changes instituted by the vendor and outside of laboratory control) will be assessed to determine if the change adversely affects the laboratory analysis in which the reagent/chemical is used. This assessment will also be documented in the verification paperwork. Kit component information in the chemicals and reagents section will be updated as required when the manual is revised.
- Verification of a reagent that is only used as a component of another reagent is achieved by verifying the final preparation and does not need to be documented separately.
- Reagents used in the same procedure may be verified simultaneously. If the verification fails, the components will then need to be verified separately.
- Verification paperwork is maintained by calendar year in the LIMS and shall include the DNA Critical Reagent Verification Form, for critical DNA reagents.
- For successful verification of screening reagents, the positive and negative controls must perform as described in the Forensic Biology Casework Procedures Manual – Part 1. Reagents must be successfully verified prior to use in casework.
- For verifications that include amplification and electrophoresis, the paperwork consists of the electropherograms for the positive control/reference sample(s) and negative control/blank(s). Verification results are assessed as described in the Data Interpretation section of the Forensic Biology Casework Procedures Manual – Part 2. The expected results must be obtained for a chemical/reagent to be successfully verified and appropriate for use in casework/database analysis.
- In the verification of amplification kits, the relative fluorescence units (RFU) for the known sample amplified with the new kit are compared to the results obtained with the kit currently in use to estimate the sensitivity of the new kit. This is important for adjusting the target value with the new lot of kits.
- The central log paperwork for verifications may be referenced by noting the batch in which the verification was performed.
- Upon successful verification, the reagent log shall be updated with the verification date and scientist, and the storage location for the reagent.
- When verification fails on a reagent prepared in-house, the reagent may be re-prepared and/or verification repeated. If verification fails again, consult with the DNA Technical Manager to determine the appropriate course of action. For purchased reagents/chemicals, the DNA Technical Manager shall be consulted to determine the appropriate course of action.

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Preparation and Verification of Reagents and Chemicals

AmpliAq Gold® DNA Polymerase (DNA critical reagent)
Purchased from Life Technologies and stored at -20°C

Verification Procedure

Amplify and analyze a previously typed reference sample and a corresponding reagent blank using the new lot of polymerase.

Buffer ATL (DNA critical reagent)
(when purchased outside of a kit)
Purchased from Qiagen and stored at room temperature

Verification

Extract, amplify and analyze a previously typed reference sample and a corresponding reagent blank using the new lot of buffer.

Buffer G2 (DNA critical reagent)
(when purchased outside of a kit)
Purchased from Qiagen and stored at room temperature

Verification

Extract, amplify and analyze a previously typed reference sample and a corresponding reagent blank using the new lot of buffer.

Buffer MTL (DNA critical reagent)
Purchased from Qiagen and stored at room temperature

Verification

Extract, amplify and analyze a previously typed reference sample and a corresponding reagent blank using the new lot of buffer.

DNASTable® LD (DNA critical reagent)
Purchased from Biomatrix and stored at 2-8°C.

Verification

Use DNASTable® LD to dry down a previously typed reference sample extract (50 µL) and a corresponding reagent blank extract (50 µL). Rehydrate each of the dried extracts and amplify. Comparison of the original amplification results to the results of the rehydrated extract should not demonstrate a significant (>20%) overall reduction in the average peak heights.

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DTT (1M)**(DNA critical reagent)**Working Solution

Dissolve 0.77g dithiothreitol in 5mL sterile de-ionized water in a sterile conical tube. Add 50µL of 3M Sodium Acetate buffer solution, pH 5.2. Do not autoclave. Aliquot (0.1mL recommended) and store at -20°C. Aliquots expire one year from date of first thaw.

Verification

Extract, amplify and analyze a previously typed semen sample and a corresponding reagent blank using the new DTT lot.

EZ1 DNA Investigator Kit**(DNA critical reagent)**

Components: Reagent Cartridges, Buffer G2, Proteinase K solution, carrier RNA

Purchased from Qiagen and stored at room temperature. Reagent cartridges may be stored at 2-8°C for long-term storage

Carrier RNA solution is prepared by reconstituting the carrier RNA in 310µL of sterile, de-ionized water. Vortex and spin briefly. Prepare 20µl, single use aliquots in 0.5mL tubes and store at -20°C. Reconstituted carrier RNA expires one year from date of preparation.

Verification

Extract, amplify and analyze a previously typed reference sample and a corresponding reagent blank using all components from the new kit lot.

**Brentamine / Fast Blue B
Solution #1**

Dissolve 10 mg α -Naphthyl Phosphate in 10 mL deionized water.

Solution #2

Dissolve 2.5 mg Fast Blue B (o-Dianisidine Tetrazotized) in 10 mL sodium acetate buffer (0.14 M, pH ~5.0).

Store both solutions at 2-15°C; solution expires 7 days from date of preparation.

Alternatively, these reagents may be made in bulk, aliquotted and frozen. Frozen reagents expire one year from date of preparation; thawed aliquots expire one day from date of thaw.

Verification

Test the reagent with a positive semen control and a negative dH₂O control prior to first use, and on each day used in casework.

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Hi-Di Formamide

Purchased from Life Technologies. Aliquot (0.5mL and 1.0mL recommended) and store at -20°C. Aliquots are intended for one-time use and should not be re-frozen.

Nuclear Fast Red stain

Dissolve 5.0g of aluminum sulfate in 100ml of hot deionized water (~40°C). Add 0.1g of Nuclear Fast Red. Stir and let cool. Filter the solution and store at room temperature; expires one year from date of preparation.

One-step PSA ABACards

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions.

Verification

A known human semen standard and sample blank are to be run to verify a new lot(s) of cards. Pooled human semen is spotted onto a stain card as a mock semen stain. The sample is processed similar to a casework sample, as described in Section 2 of the Forensic Biology Casework Procedures manual (FBCP1, current version). Record the lot number(s) and expiration date(s) and test results.

One-step HemaTrace ABACards

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions.

Verification

A known human blood standard (positive control) and a negative control (extraction buffer or deionized water) are run to verify a new lot(s) of cards. Follow the test procedure described in Section 2 of the Forensic Biology Casework Procedures –Part 1. Record the lot number(s) and expiration date(s) and test results.

Permout

Purchased from a commercial vendor and stored at room temperature.

Working Solution: Permout diluted with Xylene substitute if necessary. Use until no longer functioning adequately as a mounting medium.

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Phenolphthalein (for Kastle-Meyer Test)

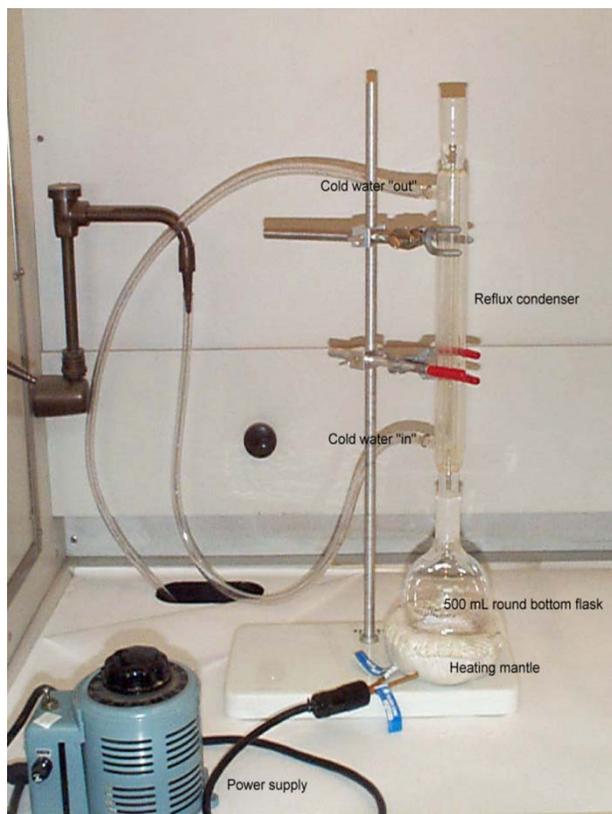
Stock Solution

Reflux 2g phenolphthalein, 20g potassium hydroxide, and 100mL deionized water with 20g of zinc until the solution becomes colorless (approximately 30 minutes to 1 hour after boiling begins – See Figure 1). Store the solution at 2-8°C in a dark bottle to which some zinc has been added to keep it in the reduced form.

Working Solution

Combine 20mL phenolphthalein stock solution (obtained from the biological screening discipline) with 80mL Ethanol (anhydrous reagent grade). The solution is stored at 2-8°C in a dark bottle. This reagent has no expiration date and may be used as long as the appropriate reactions are observed with the positive and negative blood controls, prior to use on evidentiary items.

Figure 1. Phenolphthalein Stock Solution Preparation.



- Assemble the reflux apparatus as shown.
- Turn on cold water at source. Allow the system to fill and cool. Adjust flow so that no bubbles are formed in the condenser.
- Add the chemicals, deionized water and zinc to the 500mL round bottom flask.
- Reassemble the apparatus. Place the flask on the heating mantle.
- Turn on the power supply. Heat the flask to a gentle boil (100°C for approximately 15 minutes)
- Adjust temperature setting to 75°C and allow the solution to reflux until colorless (approximately 2-3 hours).
- Store the solution with the zinc from the flask at 2-8°C in a dark bottle.
- Clean glassware with EDTA and water.

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Picro-indigo-carmin stain

Add 0.33g of Indigo Carmine dye to 100mL of saturated picric acid. Store at room temperature; expires one year from date of preparation.

PowerPlex® 16 Amplification and Typing Kit (DNA critical reagent)

Components: DNA positive control, 10X Primer Pair Mix, Gold ST*R 10X Buffer, Allelic Ladder, Internal Lane Standard (ILS 600)

Purchased from Promega Corporation. 2800M positive control DNA is diluted with 975µL sterile de-ionized H₂O, aliquotted and stored at 2-8°C. 9947A positive control DNA (remaining from older kits) is still to be stored at -20°C. Other components are stored according to manufacturer's instructions. The diluted positive control expires one year from date of dilution.

Verification

Amplify the positive control, a negative water control and a known sample twice using (1) the primers and buffer from the kit currently in use and the (2) the primers and buffer from the kit being verified, to compare amplification sensitivity and overall efficiency of amplification. Average peak height variation greater than 20% (between old and new kit) will be noted on the new reagents to alert scientists to the need to adjust target values.

Proteinase K Solution (DNA critical reagent)

(when purchased outside of a kit)
Purchased from Qiagen or another suitable vendor and stored at room temperature

Verification

Extract, amplify and analyze a previously typed reference sample and a corresponding reagent blank with the new Proteinase K lot.

QIA Symphony Investigator Kit (DNA critical reagent)

Components: Reagent Cartridges, Buffer ATL, Proteinase K solution

Purchased from Qiagen and stored at room temperature.

Verification

Extract, amplify and analyze a previously typed reference sample and a corresponding reagent blank using all components from the new kit lot.

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Quantifiler Duo Kit**(DNA critical reagent)**

Components: PCR Reaction Mix, Primer, DNA Standard, Dilution Buffer

Purchased from Life Technologies. All reagents received and stored at – 20 °C until thawed for first use. Once thawed, reagents are stored at 2-8 °C. Standard curves have an expiration date of two weeks after date of preparation.

Verification

- Test the new QD lot with a standard curve and NTCs. Expected results are defined in Section 5.6 of the Forensic Biology Casework Procedures Manual – Part 1.
- Repeat verification with a newly prepared standard curve if the defined criteria are not met with first testing and reject the kit if the test lot fails after three verification attempts.
- If the Y-intercept value observed in the new lot number differs by more than 0.5 from that observed in the previous lot number, note this difference both on the verification paperwork and the reagent storage container.
- For verification of RT-PCR reagents, the verification paperwork includes the Q-PCR set-up sheet and the Experimental Results Report.

Sodium Acetate Buffer (0.14 M, pH ~5.0)

(for FBB preparation)

Dissolve 1.2 g Sodium Acetate (anhydrous) in 100 mL deionized water. Adjust the pH to 5.0 with glacial acetic acid. Store solution at room temperature; expires one year from date of preparation.

Sterile De-ionized Water (H₂O)**(DNA critical reagent)**

Fill glass bottles with nanopure de-ionized H₂O. Autoclave (alongside a Sterikon™ plus Bio-indicator, or equivalent) for 30 minutes and store at room temperature. Expires 1 year from date prepared.

The autoclaved ampoule and a control ampoule that are placed in an incubator (at ~56°C) for 48 hours. Evaluate as per manufacturer's instructions. Seek Technical Manager guidance when the autoclaved ampoule does not perform as expected.

The DNA Technical Manager must approve use of reagents autoclaved without a Sterikon™ (or equivalent). This approval will be documented in the Reagent Log.

Verification

Amplify and analyze a previously typed reference sample (diluted with the new lot of water) along with a negative amplification control using the new water lot.

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Section 2 General Laboratory Maintenance

The Forensic Biology Staff are responsible for the housekeeping in the laboratory and for the routine maintenance of equipment and instruments. These tasks are delegated to a designated scientist. Other discipline members will assist as needed. Log sheets for maintenance and housekeeping are completed as appropriate.

- Receipt of packages and logging of chemicals/reagents.
 - Indicate date received on packing slip, initial and provide to the unit supervisor.
 - Unpack contents, label with date received and initials, store them in the proper location, record in logbook
 - If there are multiples of the same lot number (i.e. kits) then label the boxes or reagents with sequential numbers
 - Label with “needs verification” stickers and note on board that verification is required (if appropriate)
- Clean laboratories weekly, wiping down counters, computers, centrifuges, phones, door handles, etc. with 10% bleach. Each scientist is responsible for bleaching his/her own personal computer and workspace.
- UV PCR set-up hoods weekly (for at least four hours)
- Wipe down equipment/instruments weekly.
- Reboot genetic analyzer computers weekly.
- Sweep and mop floors monthly.
- Perform weekly, monthly, and semi-annual maintenance on instruments.
- Defragment instrument computer hard drives monthly. Each scientist is responsible for his/her personal computer(s).
- Put away clean laboratory dishes as needed.
- Keep both labs well stocked and inform the designated discipline purchasing agent of reagents and supplies that need to be ordered.
- Replenish reagents on genetic analyzers, as needed.
- Autoclave water as needed. Make sure that new kits/reagents are verified in a timely fashion.

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Section 3 Equipment / Instrument Maintenance

All maintenance and performance check records are maintained with the instrument, unless otherwise specified. Each calendar year, records are archived in the annual Forensic Biology case record in the LIMS.

When laboratory equipment is placed out of service for any reason, a note will be made in the equipment/instrument maintenance log (if applicable, as not all equipment has a maintenance log) and the equipment clearly marked with a note to alert scientists not to use the equipment until further notice.

Equipment/instrument manuals referenced in this section are either available online, on the laboratory network or in the designated location in the Forensic Biology discipline. Instrument manuals will be retained indefinitely. Equipment manuals are not required and do not need to be retained for equipment no longer in use.

3.1 Temperature Logs

Temperatures for refrigerators/freezer that contain chemicals, reagents and evidence are monitored electronically as a component of the laboratory security system. Temperatures for incubators are recorded by the scientists, when equipment is in use.

The discipline supervisor or DNA Technical Manager will be notified (by the lab manager or maintenance specialist) if a temperature falls outside of the acceptable range. Temperatures may be out of range following a prolonged period of the unit's door being opened. If the temperature falls outside of the acceptable range and is not corrected by a later second reading or a minor adjustment of the unit's temperature control, the DNA Technical Manager is consulted to determine a course of action.

3.2 Microscopes

Reference: <http://www.leica-microsystems.com/>
Leica DM1000/Leica DM1000 LED Operating Manual

General Instructions

- Simple dust is the number one enemy of microscopes and optical quality. When the scope is not in use, it should be covered with a plastic dust cover. Never leave a tube or an objective port open so that dust can get to the internal surfaces.

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- When cleaning of the microscope stand is required, use a clean, lint free cloth lightly moistened with water containing a small amount of mild detergent. Quickly follow the cleaning by wiping with a dry lint free cloth.
- Any residue of mounting medium or immersion oil on the stand or stage should be removed immediately after examinations are completed, using a cotton-tipped swab or cloth lightly moistened with xylene substitute. Following this solvent cleaning, the xylene substitute should be removed as quickly as possible using a clean, dry cloth. It is wise to follow the solvent removal with the above detergent cleaning.
- Before any physical contact is made with the lens surfaces (eyepieces, objectives, condenser, field diaphragm), any loose dust or debris should be blown off using compressed gas. Any stubborn dust, dirt or oil can be removed using lens cleaning fluid and a cotton-tipped swab.
- Proceed to clean the lens with a moistened swab by placing the tip at the center of the lens and working with light pressure toward the outside of the lens in a spiral motion. Immediately repeat this process using a dry swab. For very small objective lenses, the swab may be gently rotated between the thumb and forefinger while it contacts the lens. Examine the surface of the lens in reflected light for any evidence of smearing; if the surface is not completely clean repeat the process. When clean, a coated lens will have a uniform bluish color. It may be necessary to use a small amount of xylene substitute to remove oil or other mounting mediums (see above).
- Scopes should be cleaned, lubricated and aligned when necessary by a competent microscope mechanic.
- If artifacts caused by dirt are seen in the microscope image, one can locate their source in the following manner:
 - If the trouble can be eliminated by a slight adjustment of the condenser, look for the cause in the lamp bulb, lamp condenser, or filter in front of it.
 - If a change of focus control eliminates the artifact, look to the condenser or specimen itself.
 - If rotation of the objective lens causes the artifact to move, the soil is obviously on the objective. Similarly, if rotation of the eyepieces causes the artifact to move, the soiling is on the eyepiece.

Operation / Troubleshooting / Maintenance
See referenced manuals.

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3.3 Thermo Scientific Orion Star A111 Benchtop pH Meter and Electrode

*Reference: Thermo Scientific Orion Star A111 Benchtop pH Meter Reference Guide
Thermo Scientific Refillable Ag/AgCl pH Electrode User Guide*

Operating Instructions

- Prior to use in reagent preparation, prepare and calibrate the electrode as described in the referenced User Manual.
- The calibration buffer should be selected to be near the pH of the reagent being prepared.
- Calibration is recorded on the log provided at the back of this manual and is maintained with the equipment.
- Calibration records are archived annually in the LIMS.

Maintenance and Troubleshooting

- See referenced manual.

3.5 Mettler Toledo XS204 Analytical Balance

Reference: Excellence Analytical Balances XS Models Operating Instructions

Records of calibration and maintenance are retained by the laboratory Quality Manager in the Quality Assurance records. The weight set is calibrated every two years.

Semi-annual performance checks are performed approximately every 6 months, prior to the EZ1 Advanced XL performance checks. The check is recorded on the form provided at the back of this manual.

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3.6 Qiagen BioRobot EZ1 Advanced-XL

Reference: EZ1 Advanced XL User Manual

Qiagen supplementary protocol MA67 (Evaluating pipetting accuracy of the EZ1® Advanced XL using the EZ1 Advanced XL Test Card)

Qiagen supplementary protocol MA68 (Evaluating the temperature accuracy of the EZ1® Advanced XL)

Maintenance Procedures

Preventive Maintenance procedures are described in Section 6 of the EZ1 Advanced XL User Manual and recorded on the log provided at the back of this manual. Regular maintenance (6.1) is performed after each run and Daily maintenance (6.2) is performed at the end of each day the robot is in use.

Weekly maintenance will consist of UV decontamination (for 30 minutes as described in Section 5.7 of the User Manual). Weekly maintenance is not required if the instrument was not used during the week. Therefore, the first scientist to use an instrument during a given week will initial in the box provided on the log sheet.

NOTE: The instrument will give a warning when the lamp needs to be replaced. Notify the discipline supervisor if this warning is received.

O-rings will be greased (refer to section 6.3 of the User Manual) during the last week of the month (+/- one week).

Any preventive maintenance (PM) and service to the instrument, as well as the dates that an instrument is taken out of service or returned to service are also recorded.

Performance Checks

Performance checks shall be run bi-annually, regardless of whether or not service was performed on the instrument. Additionally, any instrument having PM or service

performed shall be subjected to a performance check to being used again for casework analyses. Performance checks are performed in accordance with Qiagen supplementary protocols MA67 and MA68 and are documented on the Maintenance Log.

The Maintenance Log (one page) and the form for recording the results of a performance check (four pages) are located at the back of this document.

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3.7 QIASymphony® SP

*References: QIASymphony® SP/AS User Manual - Operating the QIASymphony SP, Software version 3.5, 10/2010.
QIASymphony® SP/AS User Manual - General Description, 10/2010.*

Daily maintenance is performed at the end of each day the instrument is in use and is described in the Forensic Biology Database Procedures manual. Additional maintenance is as follows. All maintenance is recorded on the QIASymphony SP Maintenance Log provided at the back of this document.

| Type of task | Frequency | Personnel |
|---|---|---------------------------------------|
| Weekly maintenance | Once per week, if the instrument is in use, after the daily maintenance | Designated scientist |
| Monthly maintenance | Once per month, if the instrument is in use, after the daily and weekly maintenance | Designated scientist |
| Annual preventive maintenance and servicing | Once per year | QIAGEN Field Service Specialists only |

Weekly Maintenance Procedure

- Delete result files older than 10 days:
 - Press **"File Transfer"** in the "Main Menu".
 - Select the **"In/Output Files"** tab.
 - Press **"Delete Old Files"** in the command bar of the screen. A message appears asking if you want to delete files. Press **"Yes"** to delete the old files. After the files have been successfully deleted, a message will appear confirming the deletion. Press **"OK"** to confirm the message.
- Clean the touchscreen by wiping with ethanol. Then wipe with a cloth moistened with water and dry with paper towels.
- Clean the QIASymphony SP hood by wiping the surface with a soft lint-free cloth moistened with deionized water. Then wipe dry with a dry soft lint-free cloth or paper towel. **Important:** Do not use ethanol only use distilled water.
- Check the tightness of the tip adapter O-ring
 - In the "Main Menu" screen, press **"Service SP"**.
 - Select the service script **"CheckPipettingChannelORing.lua"**.
 - Press **"Start"** to start the tightness test.

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- Follow the instructions in the messages displayed on the touchscreen. When instructed to do so by the software, place an empty tip-rack containing 4 test tips into the tip rack slot given in the software message (see picture below).



Note: Do not start an inventory scan during the protocol run. When the inventory scan message appears, press “**No, nothing changed**”.

- If after running the tightness test for a particular tip adapter, a message is displayed with “Failed”, the O-ring must be changed. If one tip-adapter fails the tightness test, we recommend changing all 4 O-rings at the same time.
- Replace the tip-adapter O-ring if necessary
 - Reference 9.5 Maintenance of the tip adapter O-ring in the QIASymphony® SP/AS User Manual — General Description for instructions.

Monthly Maintenance Procedure

- Replace the tip-adapter O-rings
 - Reference Section 9.5 Maintenance of the tip adapter O-ring in the QIASymphony® SP/AS User Manual - General Description for instructions.

Performance Checks

Performance checks shall be run annually, regardless of whether or not service was performed on the instrument. The annual performance check consists of preventive maintenance performed by the vendor, followed by a run of 7 previously typed reference samples and a corresponding reagent blank. The samples are carried through the entire

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database analysis procedure to generate DNA profiles. All samples must yield the expected result; to include complete, concordant profiles for the previously typed samples. If this does not occur, the Technical Manager will be consulted to determine the appropriate course of action.

In addition to the annual performance check, any instrument having service performed shall be subjected to a performance check prior to being used again for reference sample analyses.

The performance check is documented on the QIASymphony Maintenance Log provided at the back of this document. The Database Batch Worksheet is also retained with the log. The analyst shall document profile concordance. Reviewer comments and initials are not required and these columns shall be marked as not applicable or n/a.

3.8 QIAgility

References: QIAgility® User Manual, 11/2011

Daily maintenance is performed at the end of each day the instrument is in use and is described in the Forensic Biology Database Procedures manual. Weekly maintenance is performed once per week, if the instrument is in use, after the regular and daily maintenance. All maintenance is recorded on the QIAgility Maintenance Log provided at the back of this document.

Weekly Maintenance Procedure

- Remove all loading blocks and the tip ejector chute from the worktable.

Note: Reference Section 8.2.2 of the QIAgility® User Manual for instructions on how to remove and replace the tip ejector chute.

- Rinse the blocks and the tip ejector chute with ethanol and rinse with de-ionized water.
- Dry with a soft paper towel.
- Replace the blocks and the tip ejector chute.
- UV decontaminate the worktable for a minimum of 30 minutes ensuring all samples, reagents, and consumables have been removed.
 - Click the light bulb button on the top bar of the software screen.
 - Use the arrows to adjust the time.
 - Press “**Start**”. An alert window appears. Verify all conditions are met and press “**Yes**” to start.
- Document the completion of the weekly maintenance on the QIAgility® Maintenance Log.
- Switch off the QIAgility instrument.

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Performance Checks

Performance checks shall be run annually, regardless of whether or not service was performed on the instrument. In addition to the annual performance check, any instrument having service performed shall be subjected to a performance check prior to being used again for reference sample analyses.

The performance check of the QIASymphony will also serve as the performance check for the QIAgility. The performance check is documented on the QIAgility Maintenance Log provided at the back of this document. When performed as an annual performance check, documentation is retained with the QIASymphony Maintenance Log.

3.9 Applied Biosystems 7500 Real-Time PCR System

*Reference: ABI Prism 7000 Sequence Detection and Applied Biosystems 7500 Real Time PCR System User Bulletin
Applied Biosystems 7500/7500 Fast Real Time PCR System Maintenance Guide*

Maintenance Procedures

Directions for performing the checks listed below are located in *ABI Prism 7000 Sequence Detection and Applied Biosystems 7500 Real Time PCR System Maintenance Guide*. Maintenance is recorded on the Maintenance Log (located at the end of this document). **Note:** it is not necessary to perform maintenance if the instrument is not in use for the relevant time period. Record as "Not In Use" in the Maintenance Log as applicable.

Weekly

- Power off the computer controlling the 7500 instrument, then after 30 seconds, power on the computer.
- Clean the surface of the 7500 instrument with a lint-free cloth.

Monthly

- Check the lamp status. If necessary, replace the halogen lamp.
- Perform a background calibration
- Run disk cleanup and disk defragmentation.

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Semiannually

- Perform a regions of interest (ROI) calibration
- Perform a background calibration.
- Perform an optical calibration.
- Perform a dye calibration.
- Perform an RNase P instrument verification run.

As needed

- Decontaminate the 7500 instrument
- Replace the halogen lamp
- Replace the 7500 instrument fuses
- Update the Windows operating system
- Update the 7500 software.
- Check computer disk space. If necessary, archive experiment files.

Performance Checks

Performance of the instrument is monitored with each run by the use of non-template controls and a standard curve with defined quality metrics for evaluation. Any plate not meeting the defined specifications (refer to section 5.6 of the Forensic Biology Casework Procedures Manual – Part 1) is brought to the attention of the DNA Technical Manager.

Additionally, any instrument having service performed shall undergo a performance check of a standard curve and NTCs before being used again for casework/database analysis.

3.10 Applied Biosystems 9700 Thermal Cyclers

Reference: *GeneAmp® PCR System 9700 96-Well Sample Block Module User's Manual*

http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_041143.pdf

Maintenance Procedures

Regular maintenance of the 9700 includes cleaning the sample wells and the heated cover (refer to pages 16-17 of the User's Manual). The wells and cover should be cleaned during the last week of each month (+/- one week) and recorded on the Maintenance Log. Any preventive maintenance (PM) and service to the instrument, as well as the dates that an instrument is taken out of service or returned to service are also recorded

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Performance Checks

Performance checks for the 9700 thermal cyclers include the Temperature Calibration Verification Test, the Temperature Non-Uniformity Test, and the Hardware Diagnostics/System Performance Tests. Performance checks are recorded on the Maintenance Log and test results are recorded on the corresponding laboratory forms.

The Temperature Calibration Verification test is performed monthly +/- one week. The Temperature Non-Uniformity Test and the Hardware Diagnostics/System Performance Tests are performed bi-annually.

The temperature verification system (used in the 9700 performance checks) is calibrated annually by an ISO 17025 external vendor. The calibration records are maintained by the laboratory Quality Assurance manager with the Quality Assurance records.

Additionally, any instrument having PM or service performed shall be subjected to the performance check tests prior to being used again for casework/database analyses.

The maintenance log form (one page) and the performance check results pages (three pages) are provided at the back of this document.

3.11 Applied Biosystems 3500xl

References: Applied Biosystems 3500xl Genetic Analyzers Reference Guide
(http://tools.invitrogen.com/content/sfs/manuals/cms_069856.pdf)

Annual Preventive Maintenance is performed in-house by manufacturer personnel. The maintenance is recorded on the maintenance log in a binder near the instruments. The service report is also maintained with the instrument records. Additional maintenance, also recorded in the log, is described below. Instrument maintenance records are archived in the LIMS annually.

3.11.1 Maintenance to be performed as needed

- Ensure adequate levels of buffer in reservoirs
- Purge old plate records
 - Click **Library** and select **Plates** in the navigation pane. All plates stored within the library will appear on the screen.
 - Select the plates to be deleted (more than one can be selected at a time).
 - Right click the mouse and select **delete**.

Note: Do not use the purge feature to delete items in the library. Doing so will delete all items with the exception of factory stored items. Thus, all multiplex assays and protocols from other manufacturers will be deleted.

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3.11.1.1 Replacing Anode Buffer Container (ABC)

The Anode Buffer Container (ABC) must be replaced after 7 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.
- Ensure that most of the 1X buffer is in the larger side of the ABC container prior to removing the seal by tilting the container slightly.
- Place the ABC into the Anode end of the instrument, below the pump. (RFID tag will face the instrument).

3.11.1.2 Replacing Cathode Buffer Container (CBC)

The Cathode Buffer Container (CBC) must be replaced after 7 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Wipe away any condensation on the exterior of the CBC using lint free lab cloth.
- Tilt the CBC back and forth gently to ensure the buffer is evenly distributed and remove the seal.
- Ensure the top of the CBC is dry (failure to do this may result in arcing) and place the appropriate septa on both sides of the CBC.
- Install the CBC on the autosampler.

3.11.1.3 Replenishing Polymer

The polymer must be replaced after 960 samples (or 120 injections) or when it has passed the expiration date.

- Click **Maintenance** (top right of the screen). In the Maintenance Wizards screen, click **Replenish Polymer** (this will take 10 to 20 minutes to complete) and follow the prompts.
- Polymer may be replenished as part of the water wash wizard.

3.11.1.4 Replacing the Capillary Array

The capillary is replaced as needed; when indicated by poor data quality.

- The following indications may suggest that a new capillary array is required:
 - Poor sizing precision or allele calling
 - Poor resolution and/or decreased signal intensity
- In the Maintenance Wizards screen click **Install Capillary Array** (this will take 15-45 minutes to complete) and follow the prompts.

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Note: Spatial and Spectral Calibrations must be performed anytime an array is replaced. A water wash, water trap flush and performance check must also be completed to verify performance of the array.

3.11.1.5 Spatial Calibration

A spatial calibration establishes a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera. A spatial calibration must be performed when the capillary array has been replaced, the detector door has been opened, or the instrument has been moved.

Performing a Spatial Calibration

- Access the Spatial Calibration screen:
 - Click **Maintenance** and then select **Spatial Calibration** in the navigation pane.
- Under Options, select **NO-Fill** or select **Fill** to fill the array with polymer before starting the calibration.
- Select **Perform QC Checks** to enable the system to check each capillary against the specified range for spacing and intensity.
- Click **Start Calibration**.

Evaluating a Spatial Calibration

- Evaluate the spatial calibration profile to ensure that you see:
 - One sharp peak for each capillary. Small shoulders are acceptable
 - One marker (+) at the top of every peak.
 - Peaks are about the same height.
- If the results meet the above criteria, click Accept Results. If the results do not meet the above criteria, click Reject Results and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User guide, "Spatial calibration troubleshooting" page 300.
- If the results are acceptable, click **View Spatial Calibration Report**. Click **Print**, select **CutePDFWriter**, specify a name for the report (i.e. Spatial Report 3500xl 03-03-2011 SEJ) and save the file under DNA_Share in the 3500xl equipment maintenance folder.

3.11.1.6 Spectral Calibration

A spectral calibration creates a de-convolution matrix that compensates for dye overlap. A spectral calibration should be performed whenever the capillary array is changed, the CCD camera or laser are realigned or replaced, or if you see a decrease in spectral separation.

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Performing a Spectral Calibration

- In the Dashboard, Click **Start Pre-heat** at least 30 minutes prior to the start of the run.
- Ensure the consumables are not expired and adequate injections remain.
- Ensure the pump assembly is free of bubbles, run the Remove bubble wizard if needed.
- Thaw the PowerPlex® Matrix Standards. Vortex and spin briefly.
- Make a 1:10 dilution of each dye fragment by mixing 2µl of the dye fragment in 18µl of sterile de-ionized distilled water.
- A matrix standard master mix is prepared by combining the diluted dye fragments in a tube as follows:
 - Hi-Di™ Formamide 668µl
 - diluted FL Matrix Standard 8µl
 - diluted JOE Matrix Standard 8µl
 - diluted TMR Matrix Standard 8µl
 - diluted CXR Matrix Standard 8µl
- Vortex and spin briefly.
- On the 3500xl Genetic Analyzer, 24 wells of a 96 well plate are used for creating a matrix for the 24 capillaries. Load 25 µl of the matrix standard master mix into each of the 24 wells and cover with a plate septa.

Note: the software uses predetermined positions for the calibration. You cannot specify standard location on the plate. The standards must be loaded in wells A1-H3.

- Briefly centrifuge the plate containing the standards and verify that each sample does not contain bubbles and is positioned correctly in the bottom of the well.
- Denature samples at 95°C for 3 minutes then snap chill for 3 minutes.
- Place the sample plate into the plate base provided with the instrument.
- Snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Access the Spectral Calibration screen:
 - Select **Maintenance**, then click **Spectral Calibration** in the navigation pane.
- Select **96** for the number of wells in the spectral calibration plate and specify the plate location (A or B) in the instrument.

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- Select **Matrix Standard** as the chemistry standard and **Promega4dye** as the dye set.
- Select **Allow Borrowing**.
- Click **Start Run**.

Evaluating a Spectral Calibration

- Passing and failing capillaries are shown in green and red respectively. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. Up to three adjacent-capillary borrowing events are allowed.
- If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times.
- View the spectral and raw data for each capillary. Ensure that the data meet the following criteria:
 - Order of the peaks in the spectral profile from left to right blue-green-yellow-red.
 - Order of the peaks in the raw data profile from left to right red-yellow-green-blue
 - The Quality Value is ≥ 0.95 and the Condition Number is ≤ 8.5
- If the data for all capillaries meet the above criteria, click **Accept Results**.
- If any capillary data does not meet the criteria click **Reject Results** and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User guide "Spectral calibration troubleshooting" page 301.
- If the results are acceptable, click **Export Spectral Calibration Results**. Click **View Spectral Calibration Report**, click **Print**, select **CutePDFWriter**, specify a name (i.e. Spectral Report 3500xl 03-03-2011 SEJ) for the report and save the file under DNA_Share in the 3500xl equipment maintenance folder.

3.11.2 Monthly Maintenance

The water wash, water trap flush, and instrument performance check are performed as part of monthly maintenance and/or anytime an array is replaced.

3.11.2.1 Computer maintenance

- Defragment the hard drive
Start > Programs > Accessories > System Tools > Disk Defragmenter

3.11.2.2 Water Wash

- The water wash may take over 40 minutes to complete
- Click **Maintenance** (top left of screen) on the dashboard.
- Select Wash Pump and Channels to run the wizard. Follow the prompts to completion.

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Note: An empty ABC reservoir may be used instead of emptying the reservoir currently on the instrument. Simply remove from the instrument, cover, and set aside. At the completion of the Water Wash Wizard replace the ABC with the reservoir previously removed from the instrument or a new reservoir.

3.11.2.3 Water Trap Flush

- Fill the supplied 20ml Luer lock syringe with warm deionized water. Expel any bubbles from the syringe.
- 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 clockwise.
- Open the Luer fitting by grasping the body of the fitting and turning it counterclockwise approximately one-half turn to loosen.
- Flush 5ml of deionized water through the trap taking extra care not to use excessive force.
- Remove the syringe from the Luer fitting by holding the fitting with one hand while turning the syringe counterclockwise.
- Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.
- Empty the water trap waste container.

3.11.2.4 Monthly Performance Check

The monthly performance check provides for assessment of the instrument system's resolution and its ability to adequately resolve the peaks of an allelic ladder within one base pair.

- Follow the instructions in Sections 2.1 and 2.2 of the Forensic Biology Casework Procedures Manual – Part 2 to prepare the 3500xl for a run
- Prepare an allelic ladder master mix by adding the following volumes of reagents to an appropriately sized tube:
 - 15µl ILS 600
 - 30µl allelic ladder
 - 285µl Hi-Di Formamide
- Vortex the master mix and spin briefly. Transfer 11µl of the master mix to the appropriate wells (i.e. A1-H3).
- Prepare a negative control master mix as follows:
 - 15µl ILS 600
 - 285µl Hi-Di Formamide
- Vortex the master mix and spin briefly. Transfer 11µl of the master mix to the appropriate wells (i.e. A4-H6).

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- Follow the remaining instructions in Section 2 to begin the run.
- Evaluate the ILS, allelic ladders and negative controls using the criteria defined in “Interpretation of Batch Controls” in the Forensic Biology Casework Procedures Manual – Part 2. Record the results for each instrument on the appropriate spreadsheet located in the discipline share on the laboratory network.
- If the instrument does not pass (i.e. not all injections meet the defined criteria), repeat the procedure. If the instrument still does not pass, consult the DNA Technical Manager and notify discipline scientists that the instrument is offline until the issue is resolved.

3.11.3 Annual Performance Check

At least once per calendar year, the laboratory shall complete a performance check of its analytical platform(s) as follows:

- Amplify a NIST traceable positive control with each amplification kit currently online.
- Analyze following laboratory procedures for casework
- Verify as per casework interpretation guidelines
- Required documentation (as described for amplification kit verification), is retained in the annual DNA case record in LIMS.

3.12 Pipettes

Pipettes are calibrated annually (in-house by a suitable vendor) and tracked on an uncontrolled spreadsheet and in the LIMS (DNA case record) as items of evidence. The spreadsheet contains the location, serial number, date of last calibration, and calibration due date for each pipette. ISO certificates of calibration received from the vendor are archived in the LIMS.

3.13 Thermometers

Thermometers are tracked on an uncontrolled spreadsheet. The spreadsheet includes, for each thermometer, the date of calibration, in-service date, and date due for replacement. Thermometers (including the probes used in robotic performance checks) are replaced annually, or when they are found to not be measuring accurately.

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DNA Critical Reagent Verification Form

Scientist:

Date:

Lot #

Expiration Date

Amplitaq Gold (casework database)

Buffer ATL

Buffer MTL

DNASTable LD

DTT

EZ1 Kits

EZ1 Reagent Cartridges
Proteinase K
G2 Buffer
Carrier RNA

G2 Buffer

PowerPlex 16 Kit (casework database)

Gold Star Buffer
Primer Pair
Positive Control
Allelic Ladder
ILS 600

Proteinase K (casework database)

QIASymphony Investigator Kit

QIASymphony Reagent Cartridges
Buffer ATL
Proteinase K

Quantifiler Duo Kit

Standard
Primer
Rxn Mix
Dilution buffer

Sterile Water

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DNA Casework Extraction Lab Cleaning Log

| Date /Initial | Task Completed | Comments |
|---------------|---|----------|
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> QIAgility maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> QIAgility maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> QIAgility maintenance completed | |
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| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> EZ1 maintenance completed <input type="checkbox"/> QIASymphony maintenance completed <input type="checkbox"/> QIAgility maintenance completed | |

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PCR Prep Lab Cleaning Log

| Date /Initial | Task Completed | Comments |
|---------------|---|----------|
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization | |
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| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> PCR hood UV sterilization | |

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PCR Lab Cleaning Log

| Date /Initial | Task Completed | Comments |
|---------------|---|----------|
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> Genetic Analyzer maintenance completed <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> Genetic Analyzer maintenance completed <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> Genetic Analyzer maintenance completed <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> Genetic Analyzer maintenance completed <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> Genetic Analyzer maintenance completed <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> Genetic Analyzer maintenance completed <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed | |
| | <input type="checkbox"/> Floors cleaned <input type="checkbox"/> Counters and equipment bleached <input type="checkbox"/> Genetic Analyzers wiped down <input type="checkbox"/> Genetic Analyzer maintenance completed <input type="checkbox"/> Monthly computer defrag <input type="checkbox"/> Thermal cycler wells cleaned <input type="checkbox"/> Thermal cycler maintenance completed | |

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Analytical Balance Performance Check

- Check that balance is level and adjust if necessary
- Weight set should be equilibrated to room temperature prior to use
- Airflow in the room should be minimized
- Weights should be taken while the scientist is seated, without applying pressure to the counter
- The same door of the balance should be used throughout a weighing session
- Weights should be handled with the supplied tweezers and always placed in the center of the weigh pan
- The first weight should be placed and removed 3-5 times before recording the first measurement. This allows for the electronics of the balance to "warm up"
- Ensure that the measurement has stabilized prior to recording
- Ensure that the display is zeroed (while the door is closed) in between measurements
- If the balance check fails at any weight, repeat the entire test. If it fails a second time, notify the DNA Technical Manager, who will determine an appropriate course of action
- Record the following measurements

| Weight Measured | Balance Readout (X.XXXX) | Acceptable Range | Result (pass/fail) |
|-----------------|--------------------------|------------------|--------------------|
| 1g | | 0.9990-1.0010 | |
| 2g | | 1.9990-2.0010 | |
| 5g | | 4.9990-5.0010 | |
| 10g | | 9.9990-10.0010 | |

Troemner weight set (S/N 4000013561)

Date of last cal _____

Mettler Toledo XS204 Balance (S/N B208726643)

Date of last cal _____

Scientist Signature

Date Check Performed

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EZ1 Advanced-XL-___ Maintenance Log for Calendar Year _____
Alaska State Tag # _____
S/N: _____

| Week of | Task Completed (scientist initial in box) | Comments |
|---------|---|----------|
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |

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Forensic Biology General Lab Maintenance

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EZ1 ADVANCED-XL-___ PIPETTING ACCURACY TEST

Alaska State Tag # _____ S/N _____

The following performance checks are to be performed approximately every 6 months. Upon completion of the tests, record the appropriate information for the laboratory balance and thermometer used in the spaces provided.

1. The Pipetting Accuracy Test is performed using Qiagen Supplementary Protocol MA67.
2. Read the instructions completely prior to beginning the test. Perform both the 100 μ L and 500 μ L tests.
3. Record the weights in the tables below and calculate the weight differences. Acceptable values are given in Step 17 of the protocol.
4. If the robot does not pass one of these tests, repeat the test.
5. If the robot fails the test a second time, consult the Technical Manager to determine the appropriate course of action.

Pipetting 100 μ L of water

| Tube | Weight before Run (g) | Weight after Run (g) | Difference (g) | Pipetted volume (μ L) | Pass/Fail |
|------|-----------------------|----------------------|----------------|----------------------------|-----------|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 11 | | | | | |
| 12 | | | | | |
| 13 | | | | | |
| 14 | | | | | |

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EZ1 ADVANCED-XL-___ PIPETTING ACCURACY TEST

Pipetting 500 μ L of water

| Tube | Weight before Run (g) | Weight after Run (g) | Difference (g) | Pipetted volume (μ L) | Pass/Fail |
|------|-----------------------|----------------------|----------------|----------------------------|-----------|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 11 | | | | | |
| 12 | | | | | |
| 13 | | | | | |
| 14 | | | | | |

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EZ1 ADVANCED-XL-___ LEAKAGE TEST

1. The Leakage Test is performed using Qiagen Supplementary Protocol MA67.
2. Read the instructions completely prior to beginning the test.
3. Record the results in the space provided below.
4. There must be no dripping from the tips during the test.
5. If the robot does not pass this test, repeat the test.
6. If the robot fails the test a second time, consult the Technical Manager to determine the appropriate course of action.

| Tube | Tips dripped during run | Pass/Fail |
|------|-------------------------|-----------|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | | |
| 10 | | |
| 11 | | |
| 12 | | |
| 13 | | |
| 14 | | |

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EZ1 ADVANCED-XL-___ TEMPERATURE ACCURACY TEST

1. The Temperature Accuracy Test is performed using Qiagen Supplementary Protocol MA68.
2. Read the instructions completely prior to beginning the test; be sure to wait the entire 20 minutes as described in Step 7 of the protocol.
3. Record the results in the space provided below.
4. If the measured temperature is within +/- 3°C, then the accuracy is within the defined specifications.
5. If the robot does not pass this test, repeat the test.
6. If the robot fails the test a second time, consult the Technical Manager to determine the appropriate course of action.

| | Measured Temperature | Test Results (Pass/Fail) |
|------|----------------------|-----------------------------|
| 60°C | _____ | _____ |

Equipment used

Laboratory Balance

Make/Model: _____

Serial Number: _____

Last Calibration Date: _____

Thermometer

Make/Model: _____

Serial Number: _____

Last Calibration Date: _____

Scientist: _____

Date: _____

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QIASymphony® SP-___ Maintenance Log for Calendar Year _____
Alaska State Tag # _____
S/N: _____

| Day of | Task Completed (scientist initial in box) | Comments |
|--------|---|----------|
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIASymphony maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call | |

APPROVED 4/14/2014

Forensic Biology General Lab Maintenance

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Version: FBGLM 2014 R0
Status: Active

QIAgility®- _____ Maintenance Log for Calendar Year _____
Alaska State Tag # _____
S/N: _____

| Day of | Task Completed (scientist initial in box) | Comments |
|--------|---|----------|
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |
| | <input type="checkbox"/> QIAgility maintenance/performance check completed <input type="checkbox"/> daily <input type="checkbox"/> weekly <input type="checkbox"/> annual <input type="checkbox"/> service call | |

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7500-__ Real-Time PCR Maintenance Log for Calendar Year _____
Alaska State Tag # _____
S/N: _____

| Date | Task Completed (scientist initial in box) | Comments |
|------|--|----------|
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 maintenance completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> semiannual <input type="checkbox"/> other <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 maintenance completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> semiannual <input type="checkbox"/> other <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 maintenance completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> semiannual <input type="checkbox"/> other <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 maintenance completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> semiannual <input type="checkbox"/> other <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 maintenance completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> semiannual <input type="checkbox"/> other <input type="checkbox"/> service call | |
| | <input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 maintenance completed <input type="checkbox"/> weekly <input type="checkbox"/> monthly <input type="checkbox"/> semiannual <input type="checkbox"/> other <input type="checkbox"/> service call | |

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7500-__ Temperature Calibration Verification Test
Alaska State Tag # _____
S/N _____

Scientist: _____

Date: _____

- **Select Instrument > Lamp Status / Replacement**
 - **Lamp status condition:** Good / Failed / Change soon
 - **Usage (Hours):** _____
- **Region of Interest Calibration:** Passed / Failed
- **Background Calibration:** Passed / Failed
- **Optical Calibration:** Passed / Failed
- **Dye Calibration:** Passed / Failed
- **RNase P instrument verification run:** Passed / Failed

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9700-__ Diagnostics and Performance Tests

Alaska State Tag # _____
Base S/N _____
Block S/N _____

1. Turn the thermal cycler on at least one hour before performing this test
2. Use the Temperature Verification System Instrument
3. Follow the testing instructions in the thermal cycler display; additional guidance is given in the GeneAmp® PCR System 9700 96-well Sample Block Module User's Manual
4. If the thermal cycler does not pass this test, repeat the test
5. If the thermal cycler fails the test a second time, consult the Technical Manager and mark the instrument as being out of service.

Date: _____

Operator: _____

Liquid Crystal Display (Disp)

All pixels ON Pass or Fail
All pixels OFF Pass or Fail

Keypad Diagnostic (Keypad) Pass or Fail

Cool and Heat Rate Test

Heating Rate: _____ °C/sec Pass or Fail
Cooling Rate: _____ °C/sec Pass or Fail

Cycle Performance Test

Average Cycle Time: _____ sec Pass or Fail
Cycle time STD: _____ sec Pass or Fail

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9700-__ Temperature Non-Uniformity Test

Alaska State Tag # _____
Base S/N _____
Block S/N _____

1. Turn the thermal cycler on at least one hour before performing this test
2. Do the Temperature Calibration Verification Test before performing this test
3. Use the Temperature Verification System Instrument
4. Follow the testing instructions in the thermal cycler display; additional guidance is given in the GeneAmp® PCR System 9700 96-well Sample Block Module User's Manual
5. If the thermal cycler does not pass this test, repeat the test
6. If the thermal cycler fails the test a second time, consult the Technical Manager and mark the instrument as being out of service.

| | | |
|------------------------|--------------|--------------|
| Date | | |
| Tested By | | |
| Probe Serial No. | | |
| Thermometer Serial No. | | |
| | | |
| Setpoint Value | 94 °C | 37 °C |
| A1 | | |
| A12 | | |
| C4 | | |
| C9 | | |
| F4 | | |
| F9 | | |
| H1 | | |
| H12 | | |



Temperature Non-Uniformity

**Test Results
(Pass/Fail)**

94°C _____

37°C _____

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Appendix A: Revision History

| FBGLM 2014 R0 Page | FBGLM 2013 R1 page | Location | Revision made |
|--------------------|--------------------|----------------------------|---|
| 1 | 1 | Document Structure | Updated page numbers as required. |
| 3 | 3 | Section 1.3 | Removed BCIP from the list of chemicals/reagents |
| 3 | 3 | Section 1.3 | Added α -Naphthyl Phosphate [solid], Fast Blue B (o-Dianisidine Tetrazotized) [solid], and Glacial Acetic Acid [liquid] |
| n/a | 5 | Section 1.4 | Removed preparation/verification of BCIP solution |
| 6 | n/a | Section 1.4 | Added preparation/verification of Fast Blue B solution |
| 9 | 9 | Section 1.4 | Revised instructions for PowerPlex 16 to reflect that 2800M is stored refrigerated and 9947A is stored frozen. |
| n/a | 10 | Section 1.4 | Removed preparation/verification of 0.01M Sodium Acetate buffer |
| 10 | n/a | Section 1.4 | Added preparation/verification of 0.14M Sodium Acetate buffer |
| 12 | 12 | Section 3.1 | Removed "water baths" from the sentence beginning "Temperatures for incubators...". |
| n/a | 14 | Section 3.3 | Removed the entire section on the water bath and renumbered subsequent sections. |
| n/a | 36 | Forms: Temperature Logs | Removed the Water Bath temperature log |
| 39-52 | 41-54 | Forms | Added - ___ to all forms that were missing it |