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

- **ABI -** Applied Biosystems (Life Technologies).
- **Critical equipment –** Equipment that requires validation, performance check or verification. This is required prior to initial use by the Forensic Biology Section or as specified in this procedure. Critical equipment includes the EZ1 Advanced XL BioRobot, QIAgility, ABI 7500, ABI 3130XL, ABI 9700, bulb thermometers, balances, pipettors, temperature chart recorders, biosafety cabinets, chemical fume hoods, laminar flow benches, heat blocks, and refrigerators/freezers that contain critical reagents.
- **FA -** Forensic Advantage.
- **Purified dH_2O -** Water that has been deionized and then filtered to the extent that no particle larger than a nanometer is present in the water.
- **NIST Traceable -** Sample, equipment or material(s) that has been verified against a National Institute of Standards and Technology certified sample, equipment or material(s).
- **QC Check -** Quality control assessment of materials or instrumentation prior to use within the Forensic Biology Section.
- **QCO -** Refers to the DNA Quality Control Officer or their designee(s).

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 and 3130XL, 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.

5.0 Procedure

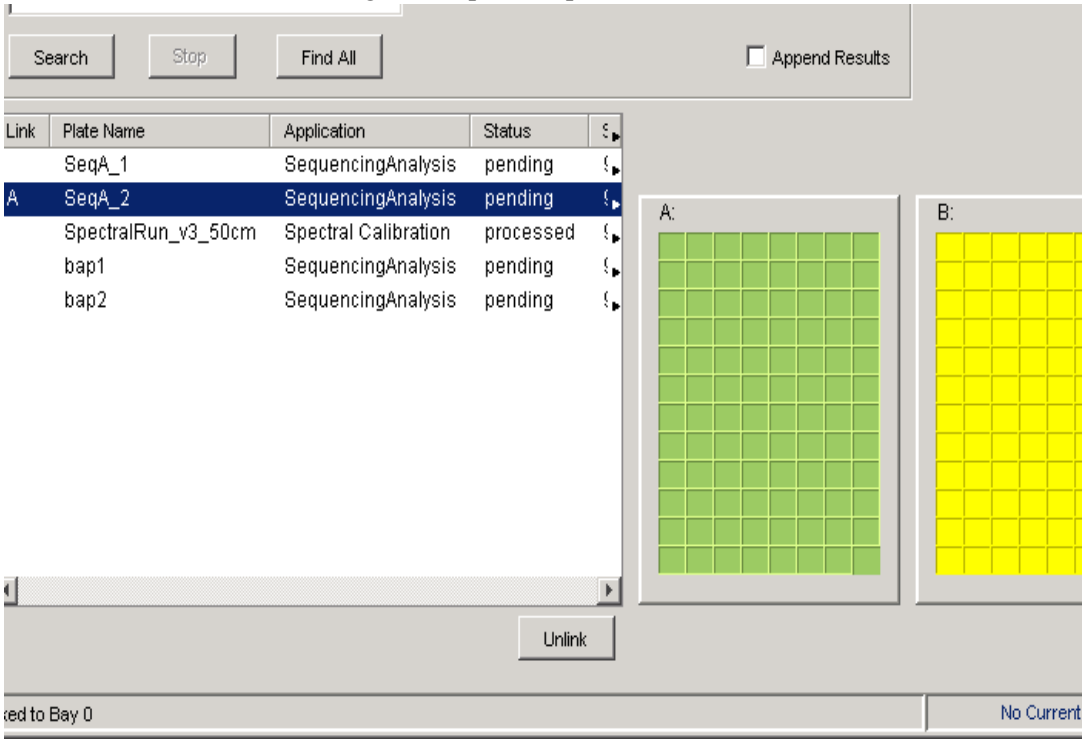
5.1 ABI 3130XL Genetic Analyzers

5.1.1 Maintenance to be Performed by First User of the Day: refer to the Forensic Biology Section Procedure for Use of the 3130XL Genetic Analyzer.

5.1.2 Weekly Maintenance

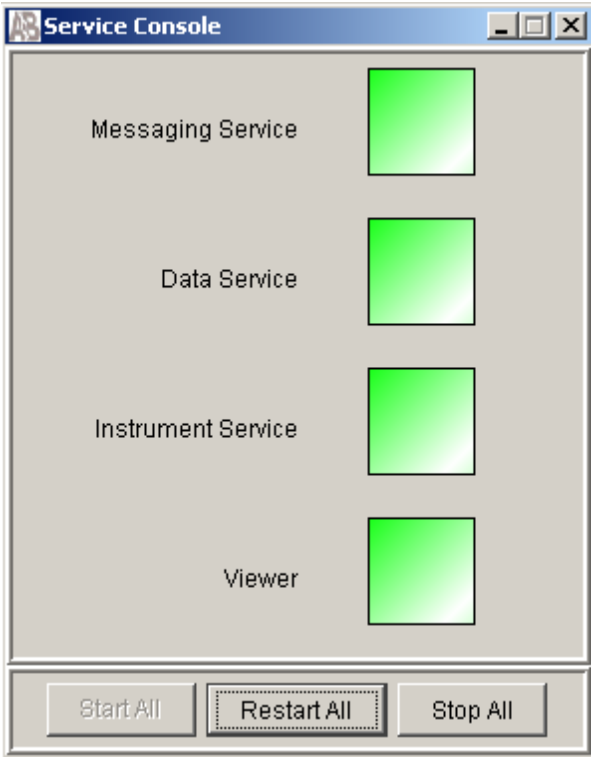
5.1.2.1 Weekly Maintenance shall be performed by the QCO. Documentation of such maintenance shall be noted on the 3130XL Monthly Maintenance Schedule chart. Such documentation shall be retained in the Section indefinitely and kept in the binder for each specific instrument which shall be located near that instrument.

5.1.2.2 At the 3130XL instrument, unlink any plates currently on the instrument. If a plate is linked, click on the green plate position and then click "unlink."



5.1.2.3 Restart the system. At the computer under service console, single click on "restart all."

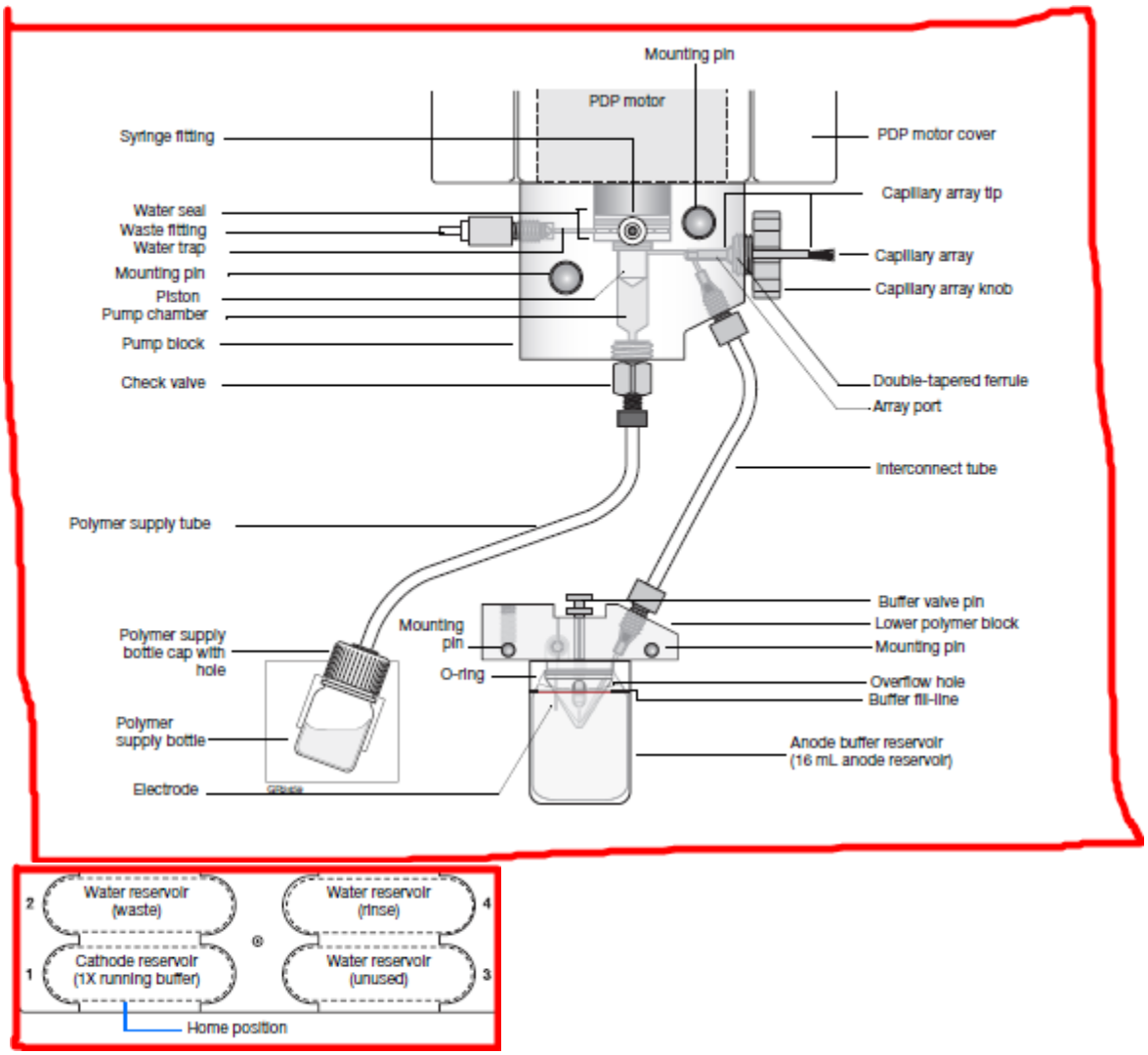
5.1.2.4 Once all areas appear as green squares, start the weekly maintenance.



5.1.2.5 Microwave ~500 mL of purified water in a beaker on high for approximately 2 minutes, until lukewarm and remove from microwave. Do NOT use boiling water.

5.1.2.6 At the 3130XL, push the tray button and wait for the autosampler tray to come to the front of the instrument. After it comes to a complete stop, and the status light is a steady green, open the doors.

5.1.2.7 Remove the anode and cathode buffer and water reservoirs and close the doors.



5.1.2.8 Discard septa in the biohazard box.

5.1.2.9 Pour contents of the reservoirs down the sink.

5.1.2.10 Using a 24 mL syringe, draw up at least 10 mL of warm purified water.

5.1.2.11 Loosen the syringe fitting and waste fitting knobs.

5.1.2.12 Place the filled (24 mL) syringe in the loosened syringe fitting knob and place the anode buffer reservoir under the waste fitting and rinse by depressing the syringe plunger slowly and steadily.

5.1.2.13 Push on the syringe and dispense at least 10 mL of lukewarm water through the syringe fitting, but do not overflow the anode buffer reservoir.

5.1.2.14 If bubbles are present in the water seal and/or water trap, re-fill the syringe and push through additional purified water until no bubbles are present.

5.1.2.15 Tighten the syringe fitting and waste fitting knobs and discard contents from the anode buffer reservoir.

5.1.2.16 Place the anode and cathode buffer reservoirs and the two water reservoirs into the purified water that was placed in microwave.

5.1.2.17 Agitate the openings and remove items from water (removing all crystals that may have formed).

5.1.2.18 Place items on a wipe and, using an additional wipe, dry the anode and cathode buffer reservoirs along with the 2 water reservoirs. Pour water down sink.

5.1.2.19 Make 1X genetic analyzer buffer from 10X stock.

5.1.2.19.1 Add 5 mL of 10X stock to 50 mL conical tube.

5.1.2.19.2 Add 45 mL purified water.

5.1.2.19.3 Invert the conical tube by hand to mix contents.

5.1.2.19.4 Label with name, date prepared, Forensic Scientist initials, and expiration date (refer to the Forensic Biology Section Procedure for DNA Reagent Preparation and Quality Control Procedure for expiration date parameters).

5.1.2.20 Add 1X buffer to the fill line of the anode and cathode buffer reservoirs and cap with a new septa. Add purified water to the fill lines of the water reservoirs and cap with a new septa as well.

5.1.2.21 At the 3130XL, push the tray button and wait for the autosampler tray to come to the front of the instrument. After it comes to a complete stop, make sure the status light is a steady green. Open the doors to the 3130XL.

5.1.2.22 Secure the anode buffer reservoir onto the 3130XL.

5.1.2.23 Place the cathode buffer reservoir and water reservoirs in the designated positions.

5.1.2.24 Check for bubbles in the polymer supply tube, interconnect tube, and array port.

5.1.2.25 If there are bubbles, click on the wizard file on the top of the Foundation Data Collection Version 3.0 Software. Click on "Remove Bubbles Wizard" and follow the prompted instructions until no bubbles remain.

5.1.2.26 Once bubbles are no longer present, check to make sure there is sufficient polymer in the polymer supply bottle.

5.1.2.27 If there is not sufficient polymer, remove the polymer supply bottle, add more polymer from the refrigerator, and re-secure the polymer supply bottle back on the instrument.

5.1.2.28 Close the instrument doors and fill in the instrument cleaning log with QCO initials, date, array usage and maintenance performed. Note any polymer information in FA (e.g., lot number, expiration date).

5.1.2.29 Change temperature chart on temperature chart recorder and label the chart with the date of the current week.

5.1.3 Water Wash

5.1.3.1 On the first Monday of each month, a water wash shall be performed by the QCO and recorded on the 3130XL Monthly Maintenance Schedule chart. Documentation shall be retained indefinitely and stored in the binder associated with the specific instrument, located near the specific instrument.

5.1.3.2 At the 3130XL instrument, make sure the plate is unlinked. If a plate is linked, click on the green plate position and then click "unlink."

5.1.3.3 Restart the Collection software: at the computer, under "service console" single click on restart all.

5.1.3.4 When all areas are green squares, start with the water wash.

5.1.3.5 Microwave ~500 mL of purified water in a beaker on high for approximately 2 minutes, until lukewarm and remove from microwave. Do NOT use boiling water.

5.1.3.6 Add at least 8 mL of purified water to the extra bottle labeled dH_2O.

5.1.3.7 At the 3130XL, push the tray button and wait for the autosampler tray to come to the front of the instrument. After it comes to a complete stop, make sure the status light is a steady green and open the doors.

5.1.3.8 Remove the anode and cathode buffer reservoirs along with the water reservoirs.

5.1.3.8.1 Discard the septa in the biohazard box.

5.1.3.8.2 Pour the contents of the reservoirs down the sink.

5.1.3.8.3 Place the anode buffer reservoir under the lower block.

5.1.3.9 On the Foundation Data Collection Version 3.0 (data collection software), click on "Wizard," highlight water wash, and run the "Water Wash Wizard."

5.1.3.10 Once the window appears, replace the water bottle with the polymer supply bottle. Do not continue until the syringe fitting knob is washed.

5.1.3.11 Using a 24 mL syringe, draw up at least 10 mL of lukewarm water.

5.1.3.12 Loosen the syringe fitting and waste fitting knobs.

5.1.3.13 Place the filled syringe in the loosened syringe fitting knob and place the anode buffer reservoir under the waste fitting.

5.1.3.14 Push on the syringe and dispense at least 10 mL of lukewarm purified water through the syringe fitting, but do not overflow the anode buffer reservoir.

5.1.3.15 If bubbles are present in the water seal and/or water trap, re-fill the syringe and push purified water through until no bubbles are present when tightening the knobs.

5.1.3.16 Tighten the syringe fitting and waste fitting knobs.

5.1.3.17 Continue to follow the instructions on the water wash wizard. Click on the "finish" button when it appears.

5.1.3.18 Place the anode and cathode buffer reservoirs and the two water reservoirs into the purified water (the following steps may be performed while the water wash wizard is running).

5.1.3.19 Agitate the opening and remove items from water (removing all crystals that may have formed).

5.1.3.20 Place items on a wipe. Using an additional wipe, dry the anode and cathode buffer reservoirs along with the two water reservoirs. Pour water down sink.

5.1.3.21 Add 1 X buffer to the fill line of the anode and cathode buffer reservoirs and cap with a new septa. Add purified water to the fill lines of the water reservoirs and cap with a new septa as well.

5.1.3.22 At the 3130XL, push the tray button and wait for the autosampler tray to come to the front of the instrument and come to a complete stop. Make sure the status light is a steady green. Open the doors to the 3130XL.

5.1.3.23 Secure the anode buffer reservoir on the 3130XL.

5.1.3.24 Place the cathode reservoirs and water reservoirs in the appropriate positions.

5.1.3.25 Check for bubbles in the polymer supply tube, interconnect tube, and array port.

5.1.3.26 If there are bubbles, click on the Wizard File on the top of the Foundation Data Collection Version 3.0 software. Then click on "Remove Bubbles Wizard" and follow the prompted instructions until no bubbles remain.

5.1.3.27 Once bubbles are no longer present, check to make sure there is enough polymer in the polymer supply bottle.

5.1.3.28 If there is not enough polymer, remove the polymer supply bottle and add more polymer from the refrigerator. Re-secure the polymer supply bottle on the instrument.

5.1.3.29 Close doors and document the maintenance as noted in **5.1.3.1**.

5.1.4 Changing the Capillary Array – when a capillary has repeated ILS (i.e., sizing standard) failure, or the bases of the alleles in samples broaden (monitor closely once the array usage approaches 200 injections), or the background noise in the electropherograms becomes repeated and excessive (based upon the training and experience of the Forensic Scientists), the array shall be replaced. Forensic Scientists shall notify the QCO and DNA Technical Leader if they observe any of the above-mentioned scenarios.

5.1.4.1 If the array is replaced at the start of the week (i.e., coinciding with weekly maintenance), the following procedure shall be followed:

5.1.4.1.1 At the 3130XL instrument, make sure the plate is unlinked. If a plate is linked, click on the green plate position and then click "unlink."

5.1.4.1.2 Restart the Collection software: at the computer under "service console," single click on "restart all."

5.1.4.1.3 When all areas are green squares, the array change may proceed.

5.1.4.1.4 Microwave ~500 mL of purified water in a beaker on high for 2 minutes until lukewarm and remove from microwave. Do NOT use boiling water.

5.1.4.1.5 Add at least 8 mL of purified water to the extra bottle labeled dH_2O.

5.1.4.1.6 At the 3130XL, push the tray button and wait for the autosampler tray to come to the front of the instrument. After the tray comes to a complete stop, make sure the status light is a steady green and open the doors.

5.1.4.1.7 Remove the anode and cathode buffer reservoirs along with the water reservoirs.

5.1.4.1.8 Discard septa into the biohazard box.

5.1.4.1.9 Pour the contents of the reservoirs down the sink.

5.1.4.1.10 Place the anode buffer reservoir under the lower block.

5.1.4.1.11 On the Foundation Data Collection Version 3.0 software, click on "Wizard." Highlight "Install Array Wizard" and follow the instructions on the software.

5.1.4.1.12 When the window appears, replace the water bottle with the polymer supply bottle stop until the syringe fitting knob is washed.

5.1.4.1.13 Using a 24 mL syringe, place it in the water and draw up at least 10 mL of lukewarm purified water.

5.1.4.1.14 Loosen the syringe fitting and waste fitting knobs.

5.1.4.1.15 Place the filled syringe in the loosened syringe fitting knob and place the anode buffer reservoir under the waste fitting.

5.1.4.1.16 Push on syringe and dispense at least 10 mL of lukewarm purified water through the syringe fitting. Do not overflow the anode buffer reservoir.

5.1.4.1.17 If bubbles are present in the water seal and/or water trap, re-fill the syringe and push purified water through until no bubbles are present when the knobs are tightened.

5.1.4.1.18 Tighten the syringe fitting and waste fitting knobs.

5.1.4.1.19 Continue to follow the instructions on "Install Array Wizard." Click on the "finish" button when it appears.

5.1.4.1.20 Place the anode and cathode buffer reservoirs and the two water reservoirs into the purified water.

5.1.4.1.21 Agitate the opening and remove items from water (removing all crystals that may have formed).

5.1.4.1.22 Place items on a wipe. Use an additional wipe to dry the anode and cathode buffer reservoirs along with the two water reservoirs. Pour water down the sink.

5.1.4.1.23 Prepare 1X buffer (see **5.1.2.19**).

5.1.4.1.24 Add 1 X buffer to the fill line of the anode and cathode buffer reservoirs and cap with new septa. Add purified water to the fill lines of the water reservoirs and cap with new septa.

5.1.4.1.25 Secure the septa on the cathode buffer reservoir along with the water reservoirs.

5.1.4.1.26 At the 3130XL, push the tray button. Wait for the autosampler tray to come to the front of the instrument and to a complete stop. Make sure the status light is a steady green.

5.1.4.1.27 Open the instrument door and place the cathode buffer reservoir and water reservoirs in their proper positions.

5.1.4.1.28 Secure the anode buffer reservoir onto the 3130XL.

5.1.4.1.29 Check for bubbles in the polymer supply tube, interconnect tube, and array port.

5.1.4.1.30 If there are bubbles, click on the Wizard File on the top of the Foundation Data Collection Version 3.0 software. Then click on "Remove Bubbles Wizard" and follow the instructions through the Wizard until no bubbles remain.

5.1.4.1.31 Once bubbles are no longer present, check to make sure there is polymer in the polymer supply bottle.

5.1.4.1.32 If polymer needs to be added, remove the polymer supply bottle and add more polymer from the refrigerator. Re-secure the polymer supply bottle back on the instrument.

5.1.4.1.33 Once complete, close doors. Fill out the 3130XL Monthly Cleaning Schedule chart with QCO initials, date, array use and maintenance performed. Any polymer or array information (e.g., changing lot numbers) shall be documented in FA.

5.1.4.1.34 Perform a Spatial Calibration (see **5.1.7**).

5.1.4.2 If the array is replaced at any other point (e.g., not coinciding with weekly maintenance), the following procedure shall be followed:

5.1.4.2.1 Push the tray button. Once the autosampler tray comes to the forward position and comes to a complete rest, make sure the status lights on the instrument are a steady green. Open the doors.

5.1.4.2.2 Click on the Wizard File on the top of the Foundation Data Collection Version 3.0 software that states "Update Cap Array Info" and follow the prompts.

5.1.4.2.3 Perform a Spatial Calibration (see **5.1.7**).

5.1.4.2.4 Note any polymer or array information in FA under the "maintenance" folder for that instrument.

5.1.5 Service and/or Repair

5.1.5.1 Repair: If a 3130XL becomes inoperable due to a need for repair by the manufacturer, 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.

Performance QC Check: if a 3130XL 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.

5.1.5.2 Annual Preventative Maintenance: the ABI 3130XL Genetic Analyzers shall have preventative maintenance performed annually by the manufacturer.

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

5.1.5.2.2 Performance QC Check: After preventative maintenance, each 3130XL shall have a post maintenance QC check performed by the QCO.

5.1.5.3 Argon-ion Laser Failure: the argon-ion laser inside the 3130XL instrument excites the dyes attached to the DNA fragments in the capillaries. When the laser fails, no fluorescent data is generated across all color channels.

5.1.5.3.1 If the argon-ion laser fails on a 3130XL instrument, the QCO shall proceed as described in **5.1.5.1**. Only the manufacturer (via field engineer) may replace the laser.

5.1.5.3.2 Once the laser has been replaced, the QCO shall perform both spatial and spectral calibrations (see **5.1.7** and **5.1.8**) if not already performed by the manufacturer during laser replacement.

5.1.5.3.3 The QCO shall then perform a Post Maintenance Performance QC Check on the instrument (see **5.1.6**).

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

5.1.5.3.5 After all conditions set in **5.1.5.3.1** through **5.1.5.3.4** 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.

5.1.5.3.6 All documentation pertaining to a laser failure shall be retained as described in **5.1.5.4**.

5.1.5.4 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.

5.1.6 Post Maintenance Performance QC Check: Before any validated 3130XL 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 3130xl 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 3130XL maintenance records. DNA TL approval shall be obtained prior to return to service of any 3130XL.

5.1.6.1 Post Annual Preventative Maintenance: If no modifications to the optical components of a 3130xl are made during annual performance maintenance (PM), the following instrument assessments shall be performed:

5.1.6.1.1 Precision: A master mix of ladder/formamide/LIZ sizing standard shall be prepared to fill a complete injection (16 wells or 2 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. The 250-bp peak within the LIZ sizing standard shall have a total range of sizes less than +/- 0.5bp and a standard deviation of less than 0.15bp.

5.1.6.1.2 Normalization: Several samples of 9947A or NIST-TS (target 1 ng based upon most recent QC results) shall be amplified and the PCR product pooled in order to fill at least three complete injections (2 complete columns per injection, including one well set aside for allelic ladder). Inject one set at normalized conditions (injection time used prior to service) to establish normalization using the most current data from other casework dedicated 3130XL instrument(s). Repeat injection times on serviced instrument with different injection times as necessary until this criterion is satisfied.

5.1.6.1.3 NIST Traceability: After precision and normalization have been successfully achieved, at least one NIST-Traceable Standard (NIST-TS) and associated Neg extraction control (see Procedure for DNA Reagent Preparation and Quality Control) shall be amplified (i.e., Identifiler™) with the appropriate amplification positive and negative control(s) and injected on the serviced instrument. The NIST-TS, positive amplification control and allelic ladder shall provide the expected allele calls at all loci tested. All negative controls shall be free of alleles.

5.1.6.1.4 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 **5.1.6.2** or place a service call to the manufacturer as necessary.

5.1.6.2 After Repair/Post PM QC Failure: After any repair to the optics system of a 3130XL (e.g., laser replacement), or if post annual maintenance QC fails, the following assessments shall be performed:

5.1.6.2.1 Precision, NIST-TS, and Normalization (see **5.1.6.1.1** thru **5.1.6.1.3**).

5.1.6.2.2 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 3130XL at previously established normalized conditions. All data within 90 to 390 bp shall be assessed for minimum threshold (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.

5.1.7 Spatial Calibrations

5.1.7.1 Purpose: establish a relationship between the signal emitted by each capillary and the position where that signal falls and is detected by the CCD camera.

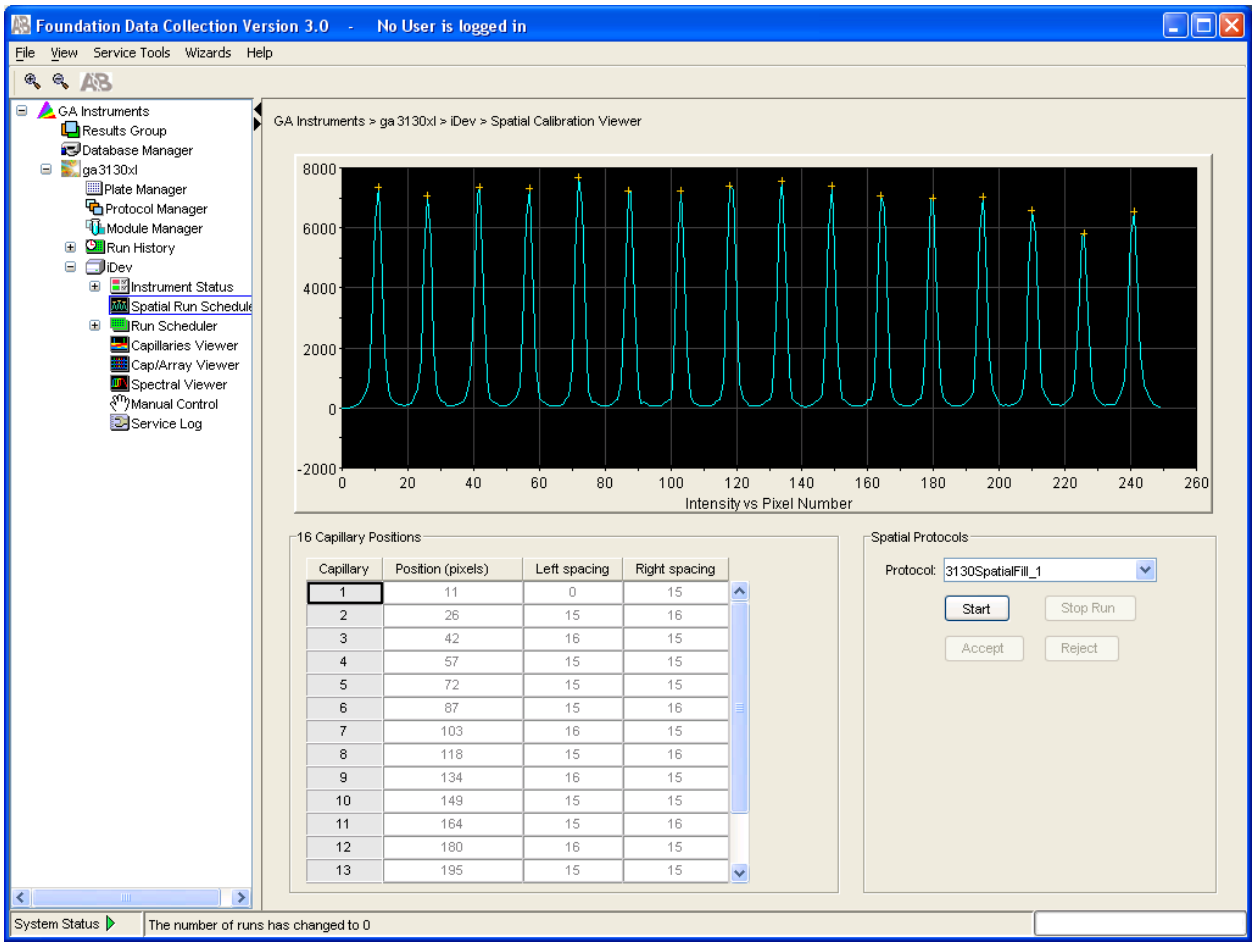
5.1.7.2 A spatial calibration shall be performed when a capillary array is installed/replaced, an instrument is moved, or when the capillary array is temporarily removed from the detection block. The spatial shall be performed by the QCO.

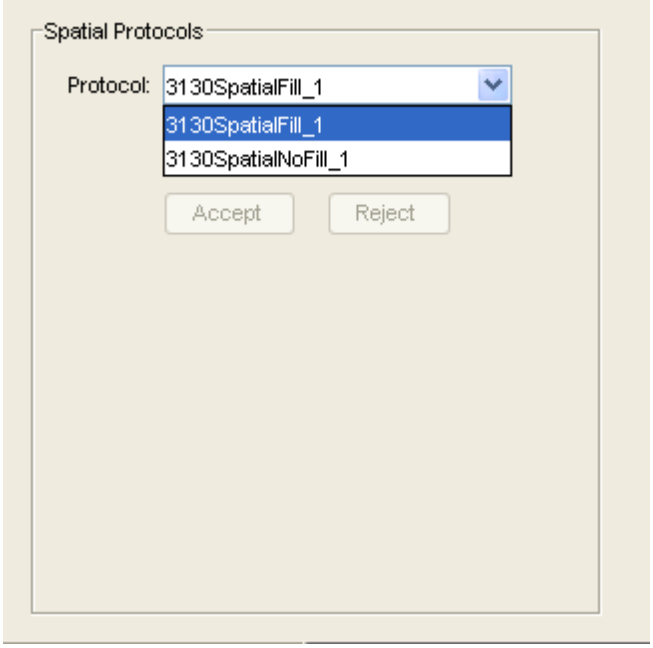
5.1.7.3 In the Data Collection software, click "ga" instruments.

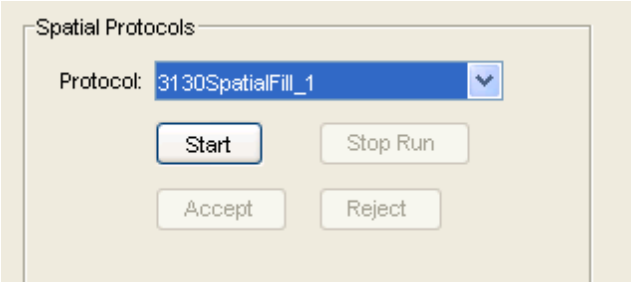
5.1.7.4 Select "ga3130" or "ga3130xl," instrument name, and spatial run scheduler.

5.1.7.5 In the spatial protocol section, select one of the following: "3130SpatialNoFill_1" or "3130SpatialFill_1." (Note: It is not necessary to fill the capillaries each time a spatial calibration is performed.)

5.1.7.6 Click start.





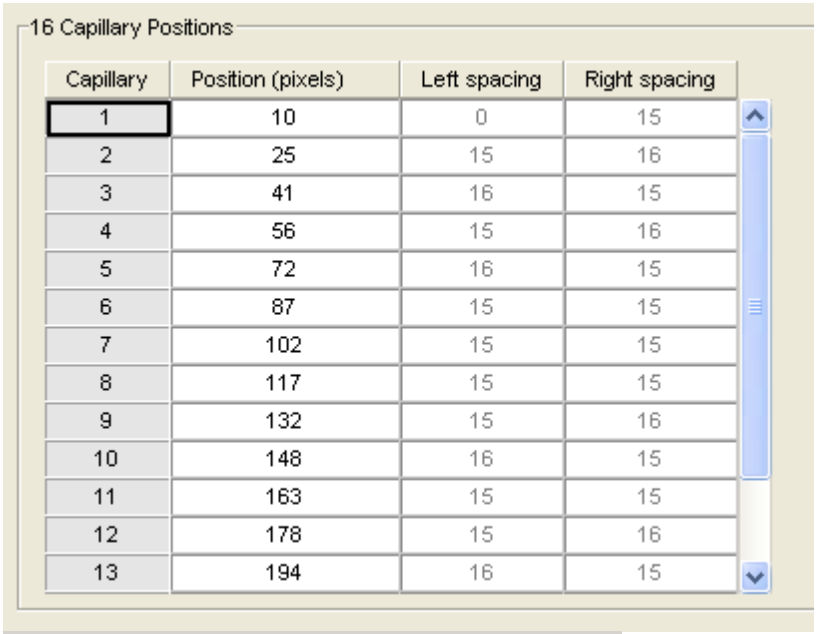


5.1.7.7 Evaluating a spatial calibration file

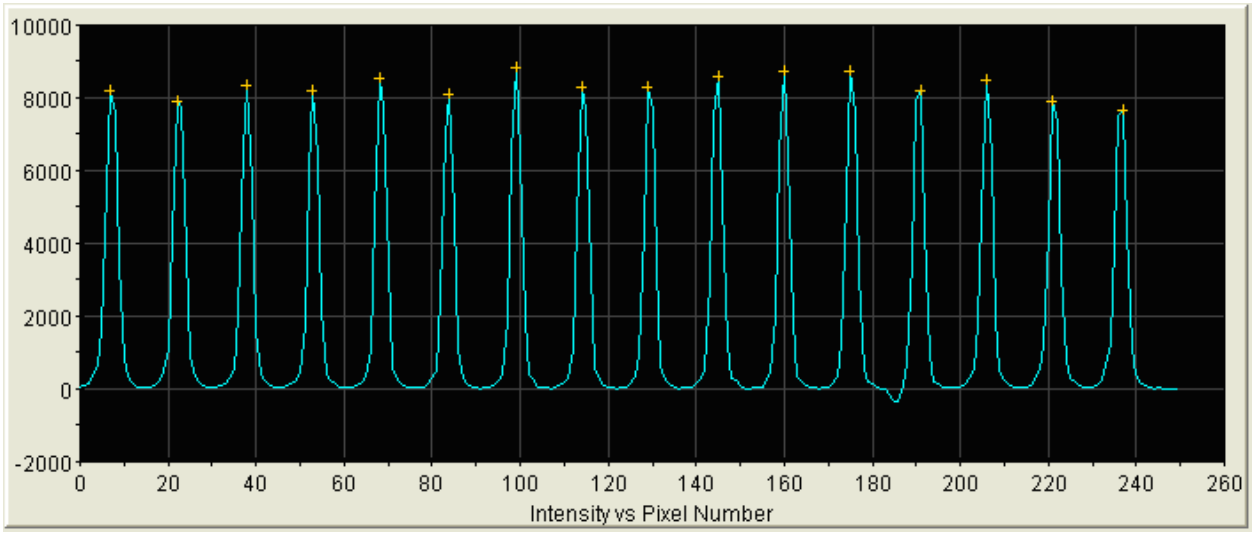
5.1.7.7.1 Ensure the following: peak heights are similar for all peaks, one orange cross marking the top of every peak (no misplaced crosses), single sharp peaks for each capillary are present with small shoulders, and the difference between adjacent positions is 13 to 16 pixels (theoretical spacing between capillaries is 15).

5.1.7.7.2 Examine each row in the 16 capillary positions table and verify that the values in both the left spacing and right spacing columns range from 13 to 16 pixels.

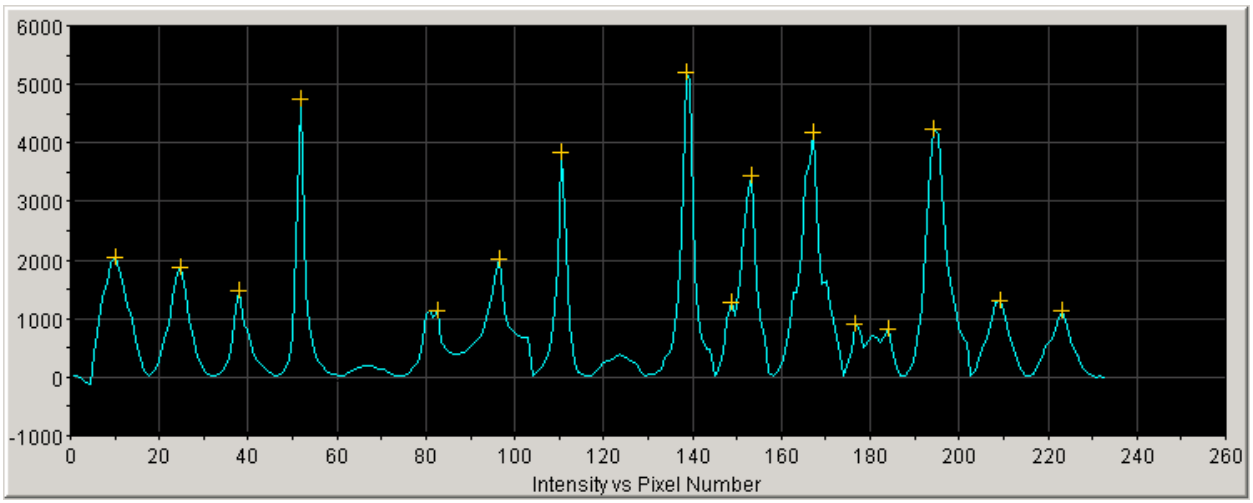
5.1.7.7.3 The spatial shall be accepted or rejected; if rejected, repeat the steps as prompted.



5.1.7.7.4 Pass:



5.1.7.7.5 Failed:



5.1.8 Spectral Calibrations

5.1.8.1 Spectral calibration creates a matrix that is used during a run to reduce raw data from the instrument to the 4-dye or 5-dye data stored in sample files.

5.1.8.2 A spectral calibration shall be performed if any of the following conditions occur: new dye set is used, the capillary array length or polymer type for fragment analysis is changed, after the laser or CCD camera has been realigned/replaced by the service engineer, if a decrease in spectral separation is seen (pull up and/or pull down peaks become excessive) in the raw or analyzed data. Note: a previously run and accepted spectral or a spectral from another instrument can be imported and used.

5.1.8.3 Remove matrix kit from the refrigerator (4 °C) and vortex.

5.1.8.4 Remove two aliquots of formamide from the freezer (-20 °C) and allow to thaw.

5.1.8.5 Add 5 µL of the matrix standard to 190 µL of Hi_Di formamide (may have to reduce to 2 µL and 198 µL based on results).

5.1.8.6 Vortex and spin briefly in a microcentrifuge.

5.1.8.7 Using a 96 well plate in columns 1 and 2 and rows A-H, add 10 µL of the matrix/formamide mixture to each well.

5.1.8.8 Cover plate with a 96 well plate septa and spin in a centrifuge for ~ 1-2 minutes @ 2000 rpm.

5.1.8.9 Remove from centrifuge and place on a thermal cycler. Denature the plate on the thermal cycler.

5.1.8.10 Assemble the plate for electrophoresis (plate base, 96 well plate, plate retainer) and press the tray button on the 3130XL to move the autosampler tray forward. Once the light stays a constant green, open the doors and place the plate on the instrument and close the doors.

5.1.8.11 Under Data Collection software, "ga instrument," "ga3130xl," protocol manager, click "new" under the instrument protocols pane and the protocol editor dialog box opens.

5.1.8.12 Complete the protocol editor dialog box (name, type: spectral, dye set, select polymer, array length, chemistry file, run module). Then click "ok." (Note: chemistry file for fragment analysis dye set defaults to the matrix standard and modules list is filtered based on the polymer type and array length.)

5.1.8.13 Under the "Data Collection software," "ga instruments," "ga3130xl," "plate manager," click "new."

5.1.8.14 Complete the new plate dialog box by entering the "plate name," "application," "plate type," "owner" (SBI), and "operator name." Click "ok."

5.1.8.15 The spectral calibration plate editor appears.

5.1.8.16 In "sample name" type in matrix and under "instrument protocol 1," select spectral.

5.1.8.17 Once the entire 2 rows are filled, click "ok."

5.1.8.18 Under "ga instrument," "ga3130xl," "instrument name," "run scheduler," click "plate view."

5.1.8.19 Change search type to advanced, "plate status" select "=," change "value 1" to "pending" and click "search."

5.1.8.20 Select the record to "run," click the plate position indicator that matches the plate to link, and click the green arrow to begin. When asked: you are about to start processing plates, click "ok."

5.1.8.21 Viewing the pass/fail status after the run:

5.1.8.21.1 Select "ga instrument", "ga3130xl," "instrument name," "instrument status," "event log."

5.1.8.21.2 In the event message section of the window, view the status of each capillary. Each capillary should have a Q-value above 0.95 (if spectral calibration failed, see troubleshooting and reference guide).

5.1.8.21.3 Under the data collection software, click "ga instruments," "ga3130xl," "instrument name," "spectral viewer."

5.1.8.21.4 In the dye set drop-down list, select the dye set.

5.1.8.21.5 In the plate diagram, select a well on the plate diagram to view the capillary spectral results (Note: a failing capillary is automatically assigned the spectral profile of its nearest passing capillary).

5.1.8.21.6 Evaluate the spectral profile and raw data for the selected capillary: verify that the order of the peaks in the spectral profile for Intensity vs. Pixel Number (from left to right) is blue, green, yellow, and red followed by orange for 5-dye chemistry.

5.1.8.21.7 Verify that the order of the peaks in the raw data profile for Intensity vs. Scan Number (from left to right) is orange, red, yellow, green, and blue.

5.1.8.21.8 Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities.

5.1.8.21.9 Repeat for each capillary in the array.

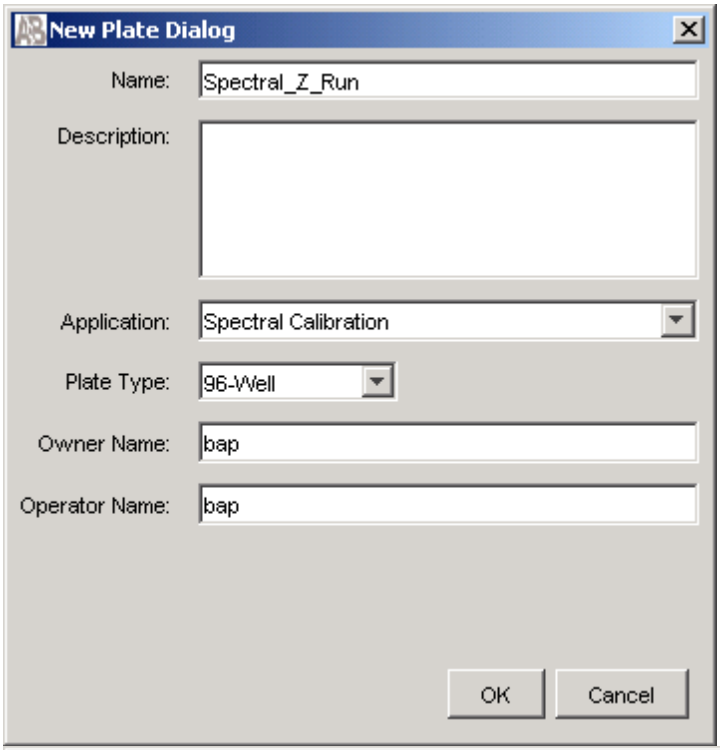
5.1.8.22 Setting an active spectral calibration:

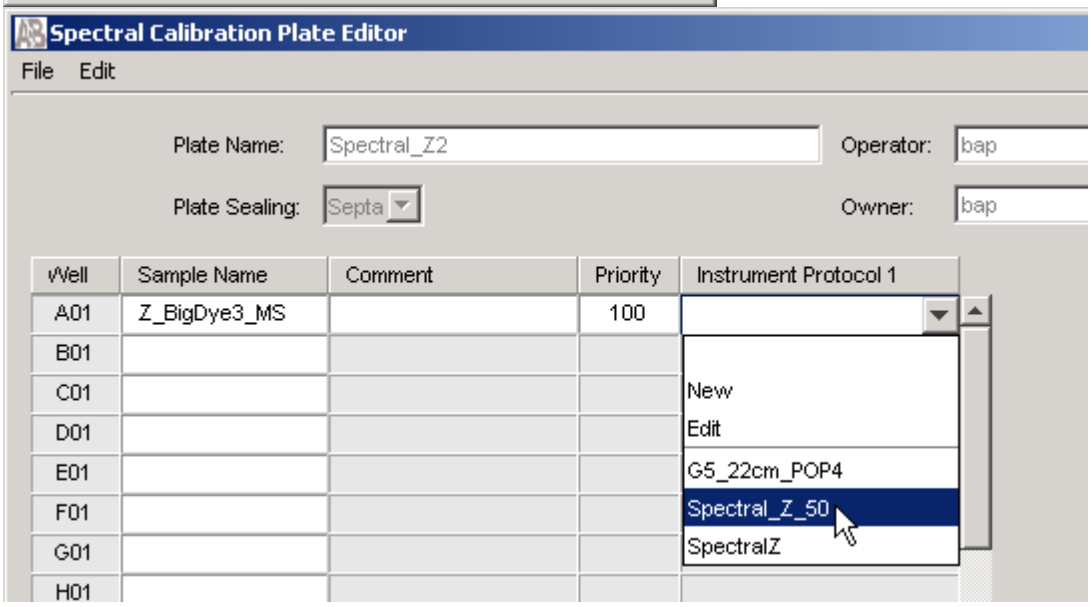
5.1.8.22.1 In the data collection software click, "ga instrument," "ga3130xl," "instrument name," "spectral viewer."

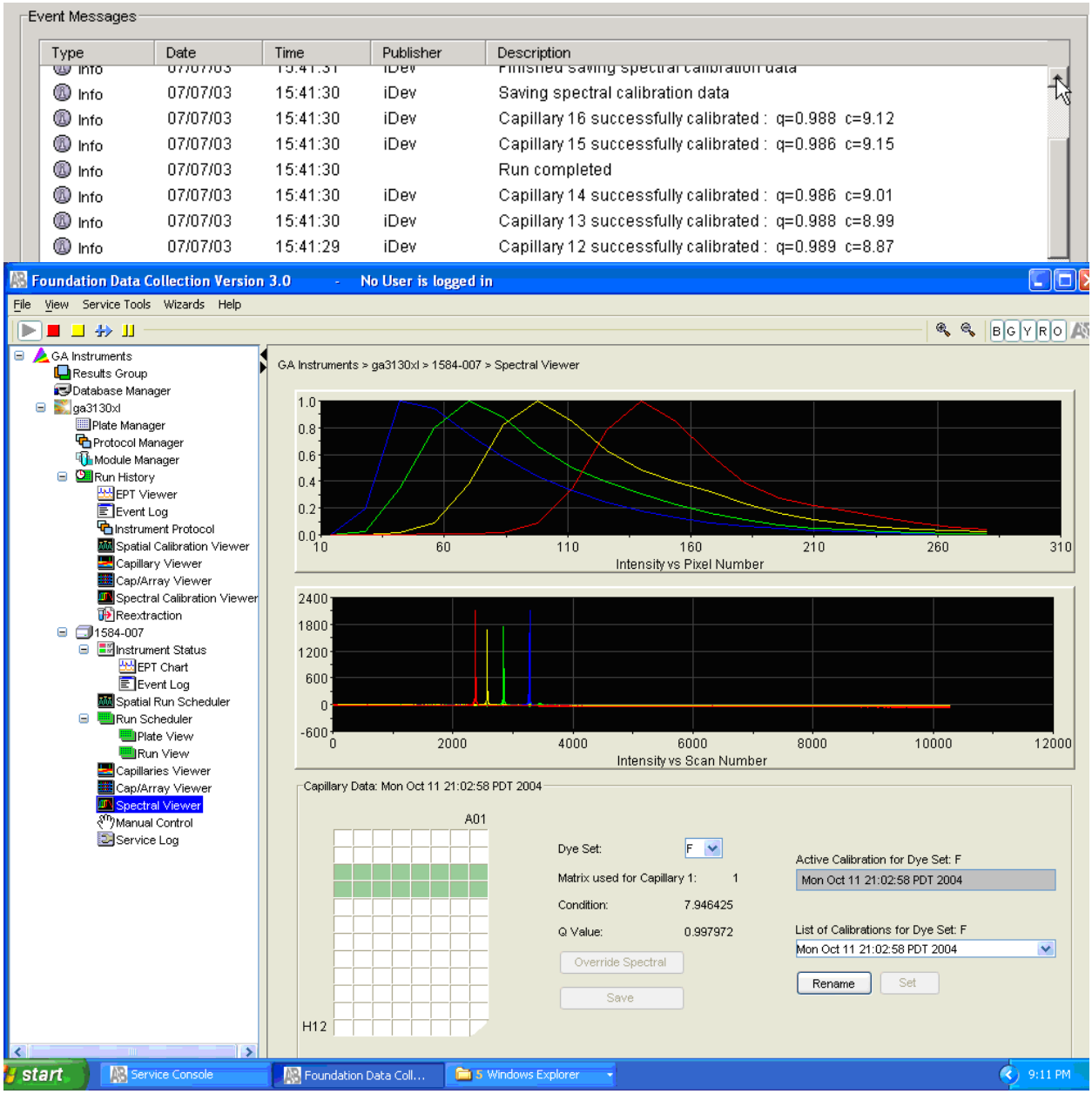
5.1.8.22.2 In the dye set drop-down list, select a dye set.

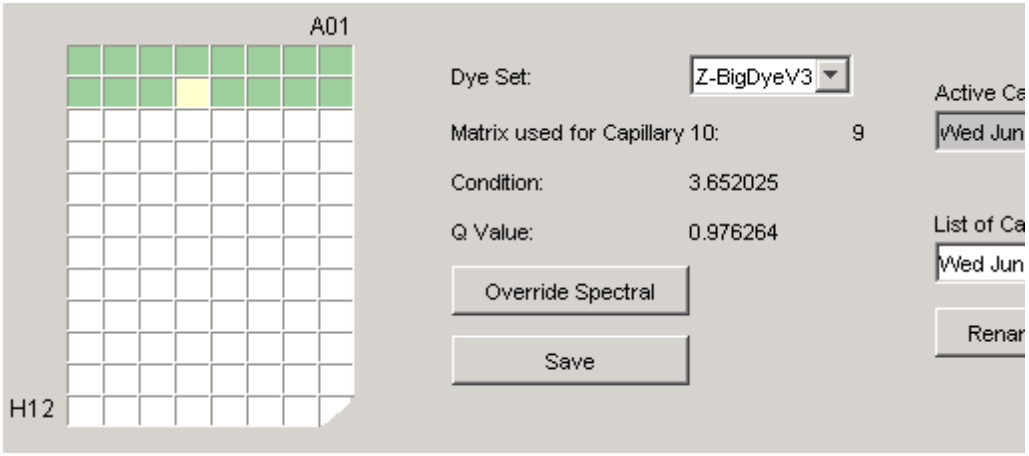
5.1.8.22.3 Select the spectral calibration to use from the dye set drop-down list.

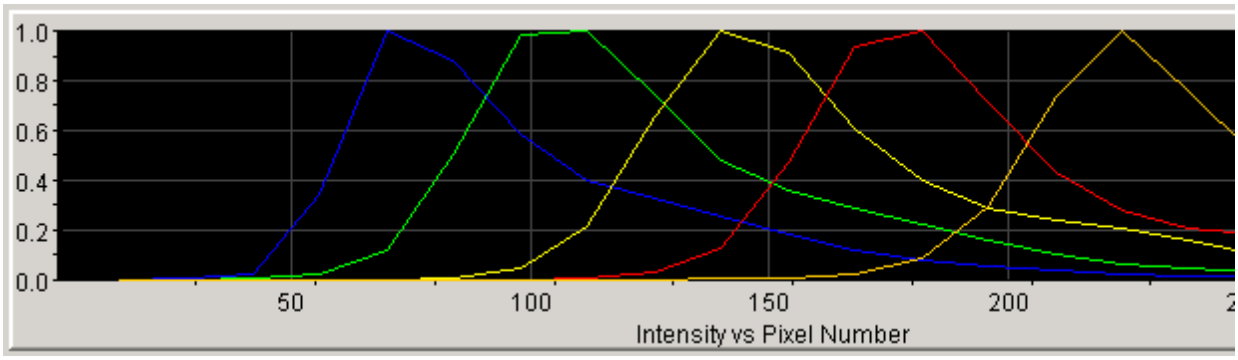
5.1.8.22.4 If the spectral calibration is acceptable, then click "set." Otherwise run a new spectral calibration.

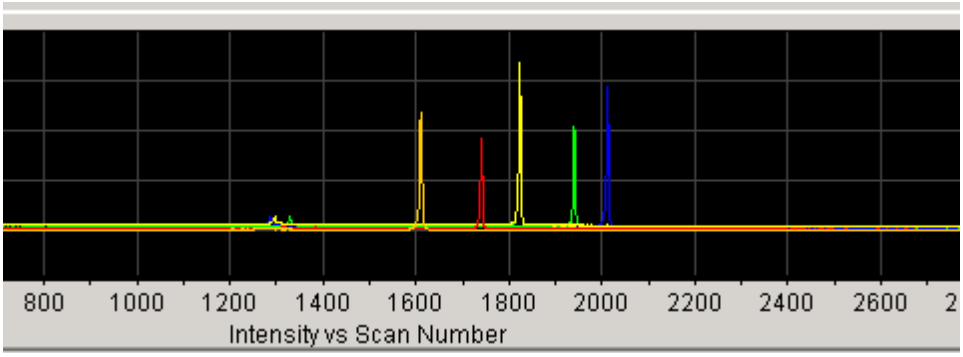












5.1.9 Clearing Errors

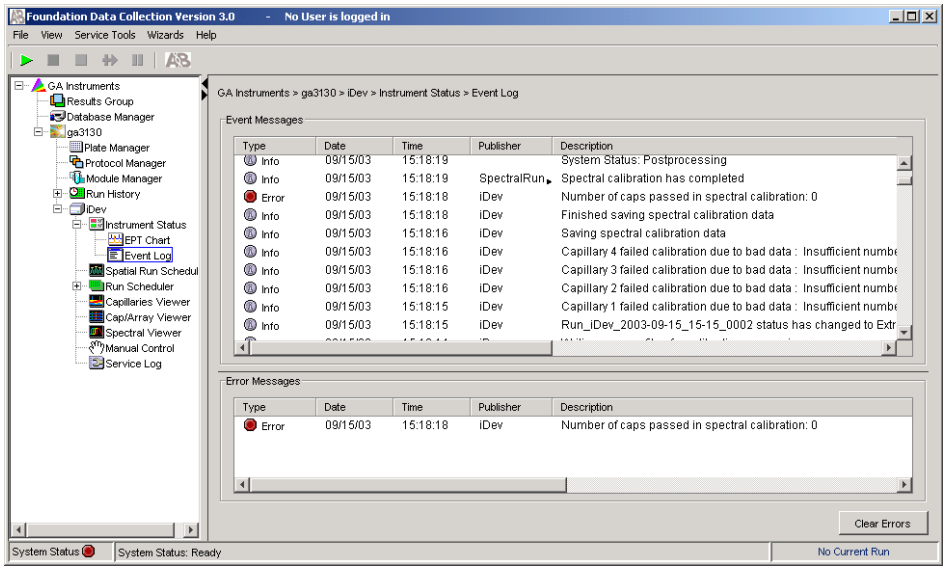
5.1.9.1 If the instrument status light is flashing red or the software System Status is flashing red, the error must be cleared before any further analysis can be completed. The QCO shall evaluate the situation and perform the following steps once notified by a Forensic Scientist that an error is present.

5.1.9.2 To clear the error on the software, open the Foundation Data Collection Version 3.0 (data collection software), under the instrument's name there is a "+" to the left of "Instrument Status."

5.1.9.3 Click the "+" to the left of "Instrument Status" and another drop down menu appears on the screen.

5.1.9.4 Click on "Event Log."

5.1.9.5 Click on the button located at the lower right that states "Clear Errors."



"Clear Errors" changes the System Status from red to green.

5.1.9.6 If the software System Status does not change back to a green arrow, or if the instrument status light does not stop flashing red, Power Off/On the Instrument (refer to the Forensic Biology Section Procedure for Use of the 3130XL Genetic Analyzer).

5.2 ABI 7500 Real-Time PCR System Instrument Maintenance

5.2.1 The following table displays the recommended 7500 instrument and laptop maintenance schedule ensuring proper operation of the instrument. Monthly, quarterly, semi-annual, and annual maintenance tasks should be performed using the listed steps/reference information at the frequencies indicated by the table. 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/archive data	---	---
Semi-annually (in order as	check lamp status (replace if needed)	---	116
	decontaminate block	---	117-120
	ROI calibration**	---	65-75

listed)	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	---

* Reference page numbers refer to the "Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Installation and Maintenance Guide" (PN# 4387777 Revision D) found in Attachment A

** The ROI calibration plate is used to perform both ROI and Optical calibration procedures. This plate, as well as the Quantifiler Duo dye calibration plates, can be used to calibrate up to three instruments and must be stored frozen, although they can remain at room temperature up to 8 hours between calibrations if protected from light exposure.

5.2.2 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.

5.2.3 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:

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

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

5.2.3.3 The NIST-TS shall indicate the presence of DNA. All testing negatives (NIST Neg K, NTC) shall have an IPC C_t value of ≥ 36 or a value stating it is "Undetermined."

5.2.3.4 Quality metrics of the standard curves (both human and male) shall fall within acceptable QC ranges.

5.2.3.5 If either **5.2.3.3** or **5.2.3.4** is not satisfied, the QCO shall repeat the QC check.

5.2.3.6 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.

5.2.3.7 The QCO shall document the testing performed and retain them in the appropriate QC files with the specific ABI 7500 maintenance records.

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 a quarterly 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 (9947A) and negative amplification control (Neg Amp), using the current amplification kit.

5.3.3.1.2 NIST-TS and associated Neg K.

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 generally between 1000 and 6000 RFU's. All Neg K and negative amplification 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 heat blocks, incubators and select refrigeration storage units. 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 the bead sterilizers and bulb thermometers purchased from an external vendor; shall be NIST traceable and replaced when NIST traceability expires.

5.8 Balances

5.8.1 Monthly Verification Check: Using a NIST traceable weight set, all balances in the Forensic Biology Section shall be verified monthly by the QCO with the following weights and limits:

5.8.1.1 Weight #1: 1 gram; Limit: 0.90 gram to 1.10 grams.

5.8.1.2 Weight #2: 25 grams; Limit: 24.90 grams to 25.10 grams.

5.8.1.3 Weight #3: 100 grams; Limit: 99.90 grams to 100.10 grams.

5.8.1.4 If any tested weight falls above or below the established limit criteria listed above, the QCO shall immediately notify the DNA Technical Leader and the balance shall be removed from service until or unless repaired and calibrated by an external vendor.

5.8.1.5 The performance checks shall be recorded to the nearest hundredth of a gram by the QCO or designee on the Monthly Balance Verification Form.

5.8.2 Biannual External Calibrations: All balances in the Forensic Biology Section shall be calibrated biannually by a contract vendor.

5.9 Pipettors

5.9.1 Biannual External Calibrations: all pipettors in the Forensic Biology Section shall be calibrated biannually 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 Chart Recorders

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

5.10.2 Biannual External Calibrations: all temperature chart recorders in the Forensic Biology Section shall be calibrated biannually by a contract vendor.

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 Heat Blocks

5.13.1 Heat blocks shall have stickers placed on them to indicate their specific purpose and associated temperature:

5.13.1.1 Knowns: 56 °C.

5.13.1.2 Unknowns: 56 °C.

5.13.1.3 Unknowns (with sperm): 37 °C.

5.13.2 The heat block temperatures shall be monitored by a calibrated bulb thermometer (see **5.5**).

5.13.3 If a Forensic Scientist uses a particular heat block for extraction, the temperature shall be recorded on the Temperature Record Form (TRF) associated with that specific heat block on the day(s) that it is used.

5.13.3.1 If the heat block is not used, the Forensic Scientist shall strike through the box which corresponds to the day(s) not in use.

5.13.3.2 The Forensic Scientist shall fill out all required information regarding equipment name and serial number, the location of the equipment, the set temperature of the equipment, and the associated bulb thermometer number.

5.13.3.3 If at any point during the calendar year a new bulb thermometer is needed, the Forensic Scientist shall write at the bottom of the TRF the date on which a new thermometer was used and the number for the new thermometer.

5.13.4 If a heat block consistently deviates more than +/- 5 °C from the set temperature for more than five consecutive readings, the Forensic Scientist shall use the temperature knob controls on the heat block to readjust the temperature back into range (this may take several attempts). If all efforts with the temperature knobs fail, the Forensic Scientist shall request a new bulb thermometer from the QCO to determine if the temperature issue is due to the heat block or the bulb thermometer. During this period of adjustment, the heat block shall not be used by the Forensic Scientist for casework purposes. If after both temperature knob adjustments and a new bulb thermometer are unsuccessful, the Forensic Scientist shall notify the QCO immediately and that particular heat block shall be removed from use. The Forensic Scientist shall note on the bottom of the TRF for that particular heat block the date it ceased to be in use.

5.14 Freezers/Refrigerators

5.14.1 Recording Temperatures: The QCO shall make every effort to record temperatures for all common area refrigerators/freezers in the Section at the beginning of every business day; 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). Refrigerators/freezers which are in limited access areas (such as between suites) shall have their temperatures recorded weekly by the Forensic Scientist who has access to such refrigerators/freezers. Temperatures are recorded on a Temperature Recording Form (TRF).

5.14.2 -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 **5.14.1**.

5.14.2.1 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.

5.14.2.2 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.

5.14.2.3 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.

5.14.2.4 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.

5.14.3 -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 **5.14.1**.

5.14.3.1 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.

5.14.3.2 If a Forensic Scientist is out of the office unexpectedly (sick day), the Manager for that Forensic Scientist shall record the temperature as required by **5.14.1**. If a Forensic Scientist has planned days out of the office (court or vacation), it is the responsibility of the Forensic Scientist to arrange for a suitemate or Manager to perform temperature recordings.

5.14.3.3 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.

5.14.3.4 If the QCO or Forensic Scientist observes temperatures out of the range specified in **5.14.3** 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 **5.14.3**, then the contents may be returned to the unit.

5.14.4 -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 **5.14.1**.

5.14.4.1 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.

5.14.4.2 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.

5.15 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.

5.15.1 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.

5.15.2 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.

5.15.3 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.

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

5.17 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.

5.18 QIAgility

5.18.1 Weekly Maintenance

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

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

5.18.1.3 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.

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

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

5.18.1.3.3 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."

5.18.1.3.4 Click Finished and then click "OK."

5.18.1.4 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.

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

5.18.2 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:

5.18.2.1 A NIST-Traceable Standard (NIST-TS) and associated Neg K (see Procedure for DNA Reagent Preparation and Quality Control) shall be quantified (e.g., Quantifiler Duo), amplified (e.g., Identifiler Plus) with the appropriate amplification positive and negative control(s) and/or setup for electrophoresis.

5.18.2.2 Items listed in **5.18.2.1** shall be electrophoresed on the 3130XL at the normalized injection protocol.

5.18.2.3 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 ≥ 36 or a value stating it is

"Undetermined." Quality metrics of the standard curves (both human and male) shall fall within acceptable QC ranges.

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

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

5.18.2.6 If **5.18.2.3, 5.18.2.4,** or **5.18.2.5** 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.

5.18.2.7 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.

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

5.19 EZ1 Advanced XL BioRobot

5.19.1 Weekly Maintenance – See the Section Procedure for DNA Extraction Using the EZ1 Advanced XL.

5.19.2 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:

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

5.19.2.2 Items listed in **5.19.1.1** shall be quantified, amplified, and electrophoresed on the 3130XL following current Section procedures.

5.19.2.3 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 ≥ 36 or a value stating it is "Undetermined." Quality metrics of the standard curves (both human and male) shall fall within acceptable QC ranges.

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

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

5.19.2.6 If either **5.19.1.3, 5.19.1.4,** or **5.19.1.5** 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.

5.19.2.7 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.

5.19.2.8 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 the 3130XL Genetic Analyzer for Casework

Forensic Biology Section Procedure for DNA Reagent Preparation and Quality Control

Forensic Biology Section Procedure for DNA Quantitation with Quantifiler® Duo

Forensic Biology Section Procedure for PCR Amplification for Casework

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 3130/3130xl 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, heat blocks (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, and Temperature Chart Recorders.
- Manufacturer documentation of preventative maintenance and/or repair for 3130XLs, ABI 7500s, centrifuges.

10.0 Attachments

A. Applied Biosystems 7500/7500 Fast Real Time PCR Maintenance Guide PN 4387777, Rev. D

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

Attachment A:

Applied Biosystems 7500/7500 Fast Real Time PCR Maintenance Guide PN 4387777, Rev. D

Maintenance Guide

Applied Biosystems

Applied Biosystems 7500/7500 Fast

Real-Time PCR Systems

System Maintenance

Maintenance Guide

Applied Biosystems

Applied Biosystems 7500/7500 Fast

Real-Time PCR Systems

System Maintenance

Part Number 4387777 Rev. D
06/2010

Contents

Preface

How to Use This Guide

Purpose of This Guide

The *Applied Biosystems 7500/7500 Fast Real-Time PCR System Guide* provides the information you need to maintain your Applied Biosystems 7500/7500 Fast Real-Time PCR System. This manual is designed to supplement the:

- Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide
- Applied Biosystems 7500/7500 Fast Real-Time PCR System Computer Setup Guide

Audience

This guide is intended for novice and experienced 7500/7500 Fast system users who need to maintain their system.

Assumptions

This guide assumes that your 7500/7500 Fast system has been installed by an Applied Biosystems technical representative and that you:

- Are familiar with the Microsoft® Windows® operating system.
- Understand general techniques for preparing and handling DNA samples.
- Have a general understanding of hard drives and data storage, file transfers, and copying and pasting.

Text Conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid user ID and password.

Safety Alert Words Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page xii.

How to Obtain More Information

Related Documentation The following related documents are shipped with the 7500/7500 Fast system:

Guide	Purpose and Audience	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Genotyping Experiments	Explains how to perform experiments on the 7500/7500 Fast system. Each Getting Started Guide functions as both: • Tutorial, using example experiment data provided with the Applied Biosystems 7500/7500 Fast Real-Time PCR Software (7500 Software). • Guide for your own experiments. Intended for laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system.	4387784
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Presence/Absence Experiments		4387785
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments		4387783
Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments		4387779
Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide	Explains how to install and maintain the 7500/7500 Fast system. Intended for laboratory staff responsible for the installation and maintenance of the 7500/7500 Fast system.	4387777
Applied Biosystems 7500/7500 Fast Real-Time PCR System Computer Setup Guide		4387778
Applied Biosystems 7500/7500 Fast Real-Time PCR System Reagent Guide	Provides information about the reagents you can use on the 7500/7500 Fast system, including: • An introduction to TaqMan® and SYBR® Green reagents • Descriptions and design guidelines for the following experiment types: – Quantitation experiments – Genotyping experiments – Presence/absence experiments Intended for laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system.	4387787

Guide	Purpose and Audience	PN
Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide	Explains how to prepare your site to receive and install the 7500/7500 Fast system. Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the 7500/7500 Fast system.	4387776
Applied Biosystems 7500/7500 Fast Real-Time PCR Software v2.0 Help	Explains how to use the 7500 Software to: • Set up, run, and analyze experiments using the 7500/7500 Fast system. • Monitor a networked 7500/7500 Fast instrument. • Calibrate a 7500/7500 Fast instrument. • Verify the performance of a 7500/7500 Fast instrument with an RNase P run. Intended for: • Laboratory staff and principal investigators who perform experiments using the 7500/7500 Fast system. • Laboratory staff responsible for the installation and maintenance of the 7500/7500 Fast system.	NA

Note: To open the user documentation included on the Documentation CD, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

Note: For additional documentation, see "How to Obtain Support" on page x.

Obtaining Information from the Help System

The 7500 software has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click ? in the toolbar of the 7500 software window
- Select **Help ▸ 7500 Software Help**
- Press **F1**

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

You can also access PDF versions of all documents in the Applied Biosystems 7500/7500 Fast Real-Time PCR System document set from the Help system.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" below).

How to Obtain Support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Safety and EMC Compliance Information

This section includes the following topics:

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard icons that are affixed to Applied Biosystems instruments* (see "Safety Symbols" on page xiii).

Examples

The following examples show the use of safety alert words:

IMPORTANT! Wear powder-free gloves when you handle the halogen lamp.

The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.

CHEMICAL HAZARD. Ethanol is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on Instruments

Electrical Symbols on Instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description	Symbol	Description
	Indicates the **On** position of the main power switch.		Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates the **Off** position of the main power switch.		Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.		Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates the **On/Off** position of a push-push main power switch.		Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety Labels on Instruments" on page xiv). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Environmental Symbols on Instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See **http://www.appliedbiosystems.com** for a list of customer service offices in the European Union.

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

English	Français
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.
WARNING This instrument is designed for 12V, 75W Halogen lamps only.	**AVERTISSEMENT** Cet instrument est conçu pour des lampes d'halogène de 12V et 75W seulement.

Locations of Warnings The Applied Biosystems 7500/7500 Fast Real-Time PCR System contains warnings at the locations shown below.

General Instrument Safety

WARNING **PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and Lifting the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and Lifting Stand-Alone Computers and Monitors

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See "About MSDSs" on page xvii.

WARNING **PHYSICAL INJURY HAZARD.** Use this instrument as specified by Applied Biosystems. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Cleaning or Decontaminating the Instrument

CAUTION Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Chemical Safety

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING **CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then click **MSDS Search**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page xvii.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING **CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical Safety

DANGER **ELECTRICAL SHOCK HAZARD.** Severe electrical shock can result from operating the Applied Biosystems 7500/7500 Fast Real-Time PCR System without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING **FIRE HAZARD.** Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

WARNING **FIRE HAZARD.** For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER **ELECTRICAL HAZARD.** Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER **ELECTRICAL HAZARD.** Use properly configured and approved line cords for the voltage supply in your facility.

DANGER **ELECTRICAL HAZARD.** Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The Applied Biosystems 7500/7500 Fast Real-Time PCR System has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical Hazard Safety

Moving Parts

WARNING **PHYSICAL INJURY HAZARD.** Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Biological Hazard Safety

General Biohazard

WARNING **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR §1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Workstation Safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.** These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian Safety Standards
- Canadian EMC Standard
- European Safety and EMC Standards
- Australian EMC Standards

U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 61010A-1, "Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements" and with standard UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

This instrument has been tested to and complies with standard CSA 1010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

Canadian EMC Standard

This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.

European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements" and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials," and with standard EN 61010-2-081:2002+A1:2003 "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

1 Overview

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

About the 7500/7500 Fast System

The Applied Biosystems 7500/7500 Fast Real-Time PCR System uses fluorescence-based polymerase chain reaction (PCR) reagents to provide:

- Quantitative detection of target nucleic acid sequences (targets) using real-time analysis.
- Qualitative detection of targets using post-PCR (endpoint) analysis.
- Qualitative analysis of the PCR product (achieved by melt-curve analysis that occurs post-PCR).

About Data Collection

The 7500/7500 Fast system collects raw fluorescence data at different points during a PCR, depending on the type of run that the instrument performs:

Run Type		Data Collection Point
Real-time runs	Standard curve	The instrument collects data following each extension step of the PCR.
	Relative standard curve	
	Comparative C_T ($\Delta\Delta C_T$)	
Post-PCR (endpoint) runs	Genotyping	The instrument collects data: • Before the PCR (For presence/absence experiments, data collection before the PCR is optional but recommended.) • (Optional) During the PCR. The instrument can collect data during the run (real-time); collecting data during the run can be helpful for troubleshooting. • After the PCR.
	Presence/Absence	

Regardless of the run type, a data collection point or *read* on the 7500/7500 Fast instrument consists of three phases:

1. **Excitation** – The instrument illuminates all wells of the reaction plate, exciting the fluorophores in each reaction.
2. **Emission** – The instrument optics collect the residual fluorescence emitted from the wells of the reaction plate. The resulting image consists only of light that corresponds to the range of emission wavelengths.
3. **Collection** – The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval, then stores the raw fluorescence image for analysis.

After a run, the 7500 software uses regions of interest (ROI), optical, dye, and background calibration data to determine the location and intensity of the fluorescence signals in each read, the dye associated with each fluorescence signal, and the significance of the signal.

Notes

About the Filters

The 7500/7500 Fast system uses five filters to support the following system dyes:

Filter 1	Filter 2	Filter 3	Filter 4	Filter 5
• FAM™ dye • SYBR® Green dye	• JOE™ dye • VIC® dye	• TAMRA™ dye • NED™ dye • CY3® dye	• ROX™ dye • Texas Red® dye	Cy5® dye

For More Information

For information on

- The 7500/7500 Fast system – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Software Help*.

  Note: To access the Help, select **Help ▸ 7500 Software Help** from within the 7500 software.

- Genotyping experiments – Refer to *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Genotyping Experiments*.
- Presence/absence experiments – Refer to *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Presence/Absence Experiments*.
- Relative standard curve and/or comparative C_T (C_T) experiments – Refer to *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Relative Standard Curve and Comparative C_T Experiments*.
- Standard curve experiments – Refer to *Applied Biosystems 7500/7500 Fast Real-Time PCR System Getting Started Guide for Standard Curve Experiments*.

Notes

How to Use This Guide

This guide describes how to maintain the Applied Biosystems 7500/7500 Fast Real-Time PCR System. Chapters 2 through 5 of this manual describe calibrations that you must perform as regular maintenance of the 7500/7500 Fast system. Chapter 6 and the appendices contain maintenance procedures that you may need to resolve infrequent problems.

Chapter/ Appendix	Title	Description
2	Perform the Regions of Interest (ROI) Calibration	Describes how to perform an ROI calibration, which allows the 7500 software to map the positions of the wells on the sample block so that it can associate the fluorescence collected during a run with specific wells of the plate.
3	Perform the Background Calibration and Optical Calibration	Describes how to perform background and optical calibrations where the: • Background calibration allows the 7500 software to remove the background fluorescence from experiment data. • Optical calibration compensates for the physical effects of the fifth filter present in 7500/7500 Fast systems.
4	Perform the Dye Calibration	Describes how to perform dye calibrations, which allow the software to distinguish the individual contribution of each dye in the total fluorescence collected by the instrument.
5	Verify the Instrument Performance	Describes how to perform a TaqMan® RNase P Instrument Verification Plate run that can be used to verify the performance of a 7500/7500 Fast system.
6	User-Performed Maintenance	Describes how to: • Replace the user-serviceable parts of the 7500/7500 Fast system. • Resolve infrequent problems that can occur during instrument use.
A	Store, Move, and Install the 7500/7500 Fast System	Describes how to store, move, and reinstall the components of the 7500/7500 Fast system.
B	Create a Custom Dye Plate	Describes how to create a dye plate that can be used to calibrate the 7500/7500 Fast system for a dye not manufactured by Applied Biosystems.
C	Create a Background Plate	Describes how to create a background plate in the event that one is unavailable.

Notes

Recommended Maintenance Schedule

The following table displays the recommended maintenance schedule for the 7500/7500 Fast instrument and computer. The procedures listed in the table are intended for the user(s) of the 7500/7500 Fast system. To ensure proper operation of your instrument, perform the regular weekly, monthly, and semiannual maintenance indicated below.

IMPORTANT! The numbered lists in the table below indicate that the tasks must be performed in sequence.

Perform...	User-Performed Maintenance Task	See Page
Weekly	• Check the computer disk space. If necessary, archive or back up your experiment files. • Power off the computer controlling the 7500/7500 Fast instrument, then after 30 sec, power on the computer. • Clean the surface of the 7500/7500 Fast instrument with a lint-free cloth. **IMPORTANT!** Do not use organic solvents to clean the 7500/7500 Fast system.	6 – –
Monthly	1. Check the lamp status. If necessary, replace the halogen lamp. 2. Perform a background calibration.‡ 3. Run disk cleanup and disk defragmentation.	58 20 6
Semiannually (6 Months)	1. Check the lamp status. If necessary, replace the halogen lamp. 2. Perform a regions of interest (ROI) calibration. 3. Perform a background calibration. 4. Perform an optical calibration. 5. Perform a dye calibration. 6. Perform an RNase P instrument verification run.	58 7 20 25 31 45
As needed	• Decontaminate the 7500/7500 Fast instrument. • Replace the Halogen Lamp. • Replace the 7500/7500 Fast instrument fuses. • Update the Windows operating system. • Update the 7500 software.	59 63 66 67 68

‡ You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must run an ROI calibration, a background calibration, an optical calibration, a dye calibration, and an RNase P instrument verification run.

Notes

Maintain the Computer Hard Drive(s)

When to Defragment and Clean Up the Hard Drive

- At least once every month
- When a message is displayed by the Windows operating system instructing you to defragment

For More Information

In the desktop, select **Start ▸ Help and Support** to access the Help for the Windows operating system. Use the search function of the Help to find information on the "Disk Cleanup" and "Disk Defragment" utilities.

IMPORTANT! Do not run the disk management utilities and 7500 software at the same time.

Archive and Back Up EDS Files

Archive EDS Files Regularly

To conserve space on the computer hard drive, older EDS files can be archived using a data compression utility. Several commercially available compression utilities are available. PKZIP and *.arc are archive formats common to the Microsoft® Windows® operating system.

Back Up EDS Files

Applied Biosystems strongly recommends that you back up your experiments.

Backing up data:

- Protects against potential loss of data caused by an unforeseen failure of the computer or its hard drive(s).
- Conserves space on the hard drive and optimizes performance, if you remove old data after backing up.

Develop a Data Management Strategy

Applied Biosystems recommends developing a strategy for managing the files produced by the 7500 software.

Note: Real-time runs generate significantly more data than genotyping or presence/absence experiments. During one day of real-time operation, the 7500/7500 Fast system can generate more than 10 MB of data.

Check Disk Space

If you perform real-time experiments on your 7500/7500 Fast system, check the amount of available space on your hard drive weekly. When the hard drive is within 20% of maximum capacity, transfer the older data to a backup storage device.

Notes

2 Perform the Regions of Interest (ROI) Calibration

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking ? in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Overview

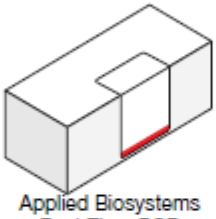
A regions of interest (ROI) calibration maps the positions of the wells on the sample block of the Applied Biosystems 7500/7500 Fast Real-Time PCR System. The 7500 software uses the ROI calibration data to associate increases in fluorescence during a run with specific wells of the plate. The instrument uses a set of optic filters to distinguish the fluorescence emissions gathered during runs. You must generate a calibration image for each individual filter to account for minor differences in the optical path.

Note: The ROI calibration is a user-performed maintenance procedure.

Time Required

30 min

Materials Required


Applied Biosystems Real-Time PCR System Spectral Calibration Kit


ROI Calibration Plate


Powder-free gloves


Safety goggles


Centrifuge with plate adapter

When to Perform the Calibration

Perform an ROI calibration:

- When installing the 7500/7500 Fast system. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Every 6 months, or as often as necessary, depending on instrument use.
- After replacing the lamp.

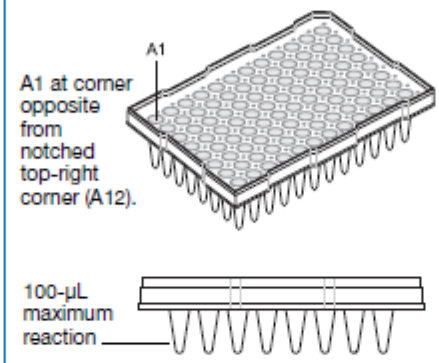
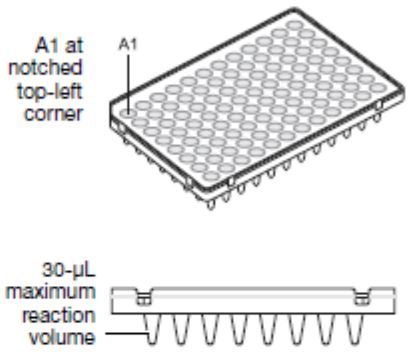
IMPORTANT! After every ROI calibration, you must perform a background calibration, optical calibration, dye calibration, and instrument verification.

Notes

Prepare the ROI Calibration Plate

Standard Plates Versus Fast Plates

Use the plate appropriate for your 7500/7500 Fast system.

Standard Plates – 7500 Systems	Fast Plates – 7500 Fast Systems
Notched corner at top right  **Vortex** standard plates to ensure complete mixing, then **centrifuge** to ensure that all reagents are contained in the bottom of the well. **CAUTION** Optical 96-Well Fast Plates do not fit correctly into the standard block.	Notched corner at top left  **Centrifuge** Fast plates to ensure that all reagents are in the bottom of the well. **Do not vortex** Fast plates. Vortexing can cause loss of smaller reaction volumes. **CAUTION** Standard plates are not compatible with the 7500 Fast system and may be crushed by the 96-Well Fast Block.

Prepare the Plate

IMPORTANT! Wear powder-free gloves when you handle the ROI calibration plate.

1. Obtain the ROI calibration plate from the spectral calibration kit in the freezer.
2. Allow the ROI calibration plate to warm to room temperature (approximately 5 min).

IMPORTANT! Do not remove an ROI calibration plate from its packaging until you are ready to run it. The fluorescent dye in the wells of the plate is photosensitive. Prolonged exposure to light can diminish the fluorescence from the plate.

Notes

3. Remove the ROI calibration plate from its packaging. Leave the optical film on the plate.

IMPORTANT! Do not discard the packaging for the ROI calibration plate. The plate can be used up to three times if it is stored in its original packaging sleeve.

4. (Standard plates only) Vortex the ROI calibration plate for 5 sec.

IMPORTANT! Do not vortex Fast plates.

(The remaining steps apply to both standard and Fast plates.)

5. Centrifuge the plate for 2 min at less than 1500 rpm.

IMPORTANT! The ROI calibration plate must be well mixed and centrifuged.

6. Verify that the liquid in each well of the ROI calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Correct

Liquid is at bottom of well.

Incorrect

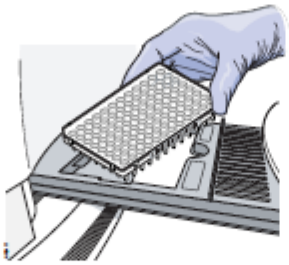
- Not centrifuged with enough force, *or*
- Not centrifuged for enough time

Notes

Load the Plate

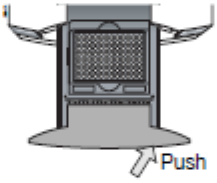
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.



Load standard plates (7500 system) with the notched A12 position at the top-right of the tray.	Load Fast plates (7500 Fast system) with the notched A1 position at the top-left of the tray.
	

3. Close the tray door. Apply pressure to the right side of the tray door at an angle.



Notes

Perform the Calibration

Automated Versus Manual Calibrations

The 7500 software allows you to perform the ROI calibration automatically or manually.

ROI Calibration	Description	Use for...	See Page
Automatic	(Novice users) A wizard interface guides you through the calibration.	Regular calibrations	12
Manual	(Advanced users) An interface similar to previous versions of the software that allows you to perform the calibration manually.	• Regular calibrations • Custom dye calibration (see page 75)	13

Perform an Automated ROI Calibration

Start the Calibration

1. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the ROI screen of the Instrument Maintenance Manager, click **Start Calibration.**
3. Complete the calibration as instructed by the wizard.

   The ROI Calibration dialog box displays three tabs:
   - **Setup** – Displays instructions for setting up the ROI calibration. Clicking Next prompts opens the Run tab.
   - **Run** – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the calibration status (Passed/Failed).

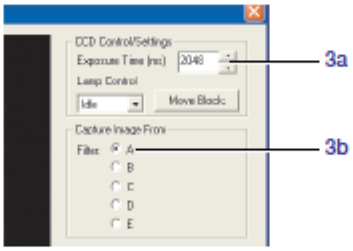
   If you cannot obtain a passing calibration, see "Troubleshoot the ROI Calibration" on page 17.

Notes

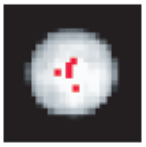
Perform a Manual ROI Calibration

Start the Calibration

1. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the ROI tab of the Instrument Maintenance Manager, click **Start Manual Calibration.**
3. In the ROI Inspector dialog box:
   a. In the Exposure Time field, enter **2048**.
   b. Select **Filter A** (Filter 1).



4. Click **Snapshot** to generate an ROI image.
5. Determine if your ROI image is acceptable (the figures below show unsaturated and oversaturated images). Wells in an acceptable image:
   - Must be as bright as possible without oversaturating. (When you generate the ROI calibration, a warning is displayed if wells are oversaturated.)
   - Can contain some, but do not have to contain any, red pixels, which represent saturation.

Unsaturated	Normal Saturation	Over Saturated
		
Good	Acceptable	Not Acceptable

6. If your ROI image is acceptable, go to step 7.

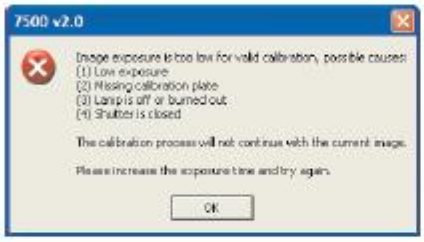
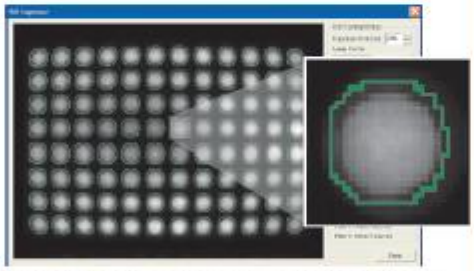
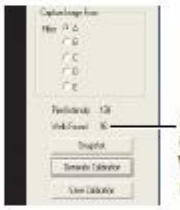
   If your ROI image is oversaturated, decrease the Exposure Time by half, then click **Snapshot**. Repeat until you obtain an acceptable ROI image.

   If you cannot obtain an acceptable image, see "Troubleshoot the ROI Calibration" on page 17.

Notes

7. Click **Generate Calibration**.

   The 7500 software takes a snapshot, then displays a message box or an ROI image:

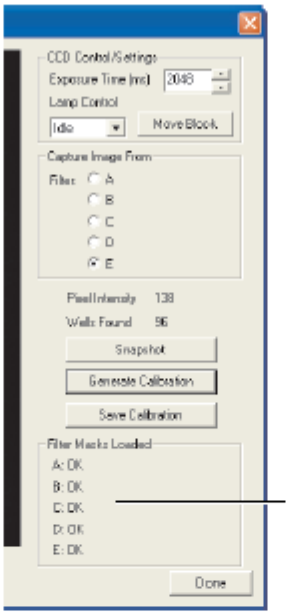
If the software displays:	Do the following:
 *or* 	a. Click **OK**. b. Decrease the Exposure Time by half (see step 3a on page 13). c. Repeat steps 3 and 5.
A very faint ROI image, or you cannot generate a successful calibration.	See "Troubleshoot the ROI Calibration" on page 17.
An acceptable Calibration Image **Note:** Green circles appear around all wells indicating that the wells calibrated successfully.  **Note:** A green circle indicates that the region of interest for the well position is bright enough.	Verify that the: • Wells Found value is 96.  • ROI Image displays a green circle around each well area.

8. Click **Save Calibration**.

   The software saves the newly generated ROI calibration for Filter 1. "OK" appears next to Filter 1 in the Filter Masks Loaded section of the ROI Inspector.

Notes

9. Repeat steps 3 through 8 for the remaining filters, resetting the Exposure Time to **2048** before performing the calibration for each filter. The ROI calibration is complete when Filter Masks Loaded for all the filters displays **OK**.



Calibration is complete when Filter Masks Loaded is **OK** for all Filters

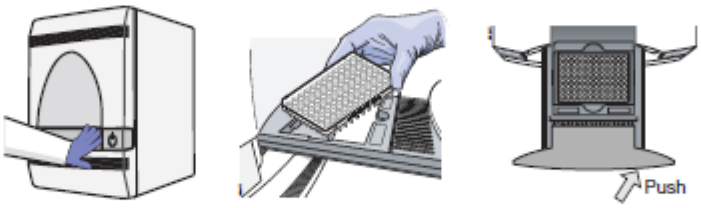
10. Click **Done**.

Notes

Unload the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Remove the calibration plate:
   a. Push the tray door to open it.
   b. Remove the calibration plate.
   c. Push the tray door to close it.



2. Place the calibration plate inside its packaging sleeve. If you plan to perform background and optical calibrations:
   - Within the next 8 hr, keep the ROI calibration plate at room temperature. The optical calibration uses the ROI calibration plate.
   - On another day, return the packaged plate to the spectral calibration kit in the freezer.

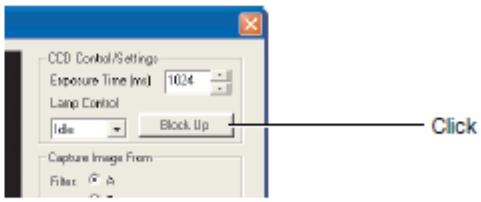
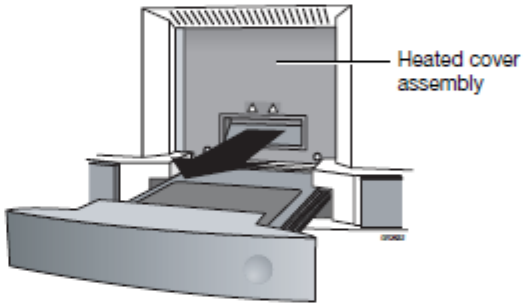
IMPORTANT! Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it up to three times after you open it.

Continue with ""Perform the Background Calibration and Optical Calibration" on page 19.

IMPORTANT! After you perform an ROI calibration, you must also perform a background calibration (see page 20), an optical calibration (see page 25), dye calibrations (see page 32), and instrument verification (see page 46).

Notes

Troubleshoot the ROI Calibration

Problem/Symptom	Possible Cause	Action
ROI Calibration Failed	The sample block may be in its lowered position.	1. If the CCD Control Settings in the ROI Inspector displays "Block Up," click **Block Up**, to raise the block.  2. Check that the heated cover assembly is pulled all the way forward to ensure that the tray can be pushed in properly. If the 7500/7500 Fast system has a heated cover latch installed, check that the latch is in a locked position.  3. If the ROI calibration continues to fail, check the status of the halogen lamp within the 7500/7500 Fast instrument (see "Monitor the Lamp Status" on page 58), then replace the lamp if necessary (see "Replace the Halogen Lamp" on page 63).
ROI Image is Faint		

Notes

Notes

3 Perform the Background Calibration and Optical Calibration

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking ? in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Perform the Background Calibration

During a background calibration, the 7500/7500 Fast system:

- Performs reads of a background plate containing PCR buffer for 10 min at 60 °C.
- Averages the spectra recorded during the run and extracts the resulting spectral component to a calibration file.

The 7500 software then uses the calibration file during subsequent runs to remove the background fluorescence from the run data.

Note: The background calibration is a user-performed maintenance procedure.

Time Required

30 min

Materials Required

Applied Biosystems 7500/7500 Fast Real-Time PCR System Spectral Calibration Kit

Powder-free gloves

Centrifuge with plate adapter

When to Perform the Calibration

Perform a background calibration:

- When installing the 7500/7500 Fast system. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Monthly, or as often as necessary, depending on instrument use.
- After replacing the lamp.

Background Fluorescence

Fluorescence data collected by the 7500/7500 Fast system includes a fluorescence signal inherent to the system, referred to as background fluorescence. Background fluorescence is a composite signal found in all spectral data. This signal consists of fluorescence from several sources, including:

- Background electronic signal
- Contaminants in the sample block
- The plastic consumable (plates and caps)

Guidelines for Calibration

- Make sure the centrifuge you use is clean. Before centrifuging, wipe down the bucket using a tissue.
- Handle the calibration plates with care to prevent contamination. Do not place plates on a lab bench, which may contaminate the plate. Always put calibration plates back into their original bags.

Notes

Prepare the Background Calibration Plate

Prepare the Plate

IMPORTANT! Wear powder-free gloves when you handle the plate.

1. Obtain the prepared background plate from the spectral calibration kit in the freezer.
2. Allow the background plate to warm to room temperature (at least 5 min).
3. Remove the background plate from its packaging.

IMPORTANT! Do not discard the packaging for the plate. The background plate can be used up to three times if it is stored in its original packaging sleeve.

4. (Standard plates only) Vortex the plate for 5 sec.

IMPORTANT! Do not vortex Fast plates.

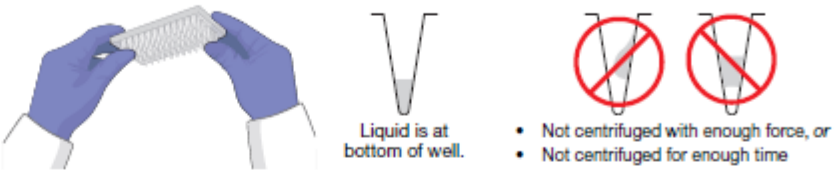
(The remaining steps apply to both standard and Fast plates.)

5. Centrifuge the plate for 2 min at less than 1500 rpm.

IMPORTANT! The plate must be well mixed and centrifuged.

6. Verify that the liquid in each well of the background plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

IMPORTANT! Do not allow the bottom of the background plate to become dirty. Fluids and other contaminants that adhere to the bottom of the plate can contaminate the sample block and cause an abnormally high background signal.

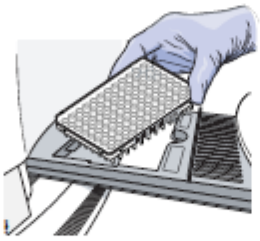


Notes

Load the Plate

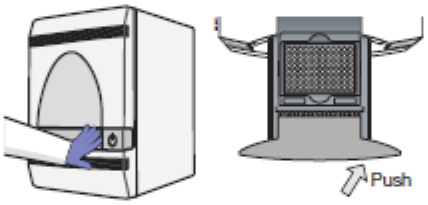
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.



Load standard plates (7500 system) with the notched A12 position at the top-right of the tray.	Load Fast plates (7500 Fast system) with the notched A1 position at the top-left of the tray.
	

3. Close the tray door. Apply pressure to the right side of the tray door at an angle.



Note: If you cannot open the tray, the sample block may be in its raised position, locking the tray door position. To lower the block, select **Instrument ▸ Calibrate**, then exit the ROI Inspector.

Notes

Perform the Background Calibration

Perform the Calibration

1. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the **Background** tab.
3. In the Background tab, click **Start Calibration.**
4. Complete the calibration as instructed by the wizard.

   The Background Calibration dialog box displays four tabs:
   - **Overview** – Displays information describing the calibration.
   - **Setup** – Displays instructions for setting up the background calibration. Clicking Next prompts opens the Run tab.
   - **Run** – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the calibration status (Passed/Failed).

   If you cannot obtain a passing calibration, see "Troubleshoot the Background Calibration" on page 30.

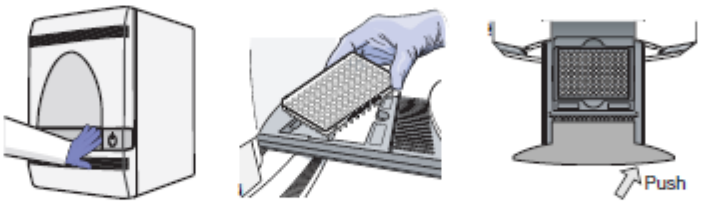
Note: Before starting the calibration, the instrument may pause (up to 10 min) to allow the heated cover to reach temperature.

Notes

Unload the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Remove the calibration plate:
   a. Push the tray door to open it.
   b. Remove the calibration plate.
   c. Push the tray door to move it into the instrument.



2. Place the calibration plate inside its packaging sleeve, then return the packaged plate to the spectral calibration kit in the freezer.

IMPORTANT! Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use the plate up to three times after you open it.

If necessary, continue with "Perform the Optical Calibration" on page 25.

You must perform an optical calibration (see page 25), dye calibrations (see page 32), and instrument verification (see page 46) if you are performing the background calibration:

- As part of your semiannual maintenance
- After replacing or moving any parts of the optics

For more information, see "Recommended Maintenance Schedule" on page 5.

Notes

Perform the Optical Calibration

The optical calibration generates data that allows the 7500 software to compensate for the physical effects of the fifth filter in the 7500/7500 Fast system.

Note: The optical calibration is a user-performed maintenance procedure.

Time Required 10 min

Materials Required ROI calibration plate

When to perform the Calibration Perform an optical calibration:

- When installing the 7500/7500 Fast system. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Every 6 months, or as often as necessary, depending on instrument use.

Prepare the Calibration Plate

Prepare the Plate If you kept your ROI calibration plate at room temperature after performing an ROI calibration (see Chapter 2), skip to step 5 on page 26 to spin down any condensation that may have formed when the plate was at room temperature. If the ROI calibration plate is in the freezer, go to step 1.

IMPORTANT! Wear powder-free gloves when you handle the plate.

1. Obtain the ROI calibration plate from the spectral calibration kit in the freezer.
2. Allow the ROI calibration plate to warm to room temperature (at least 5 min).
3. Remove the ROI calibration plate from its packaging.

IMPORTANT! Do not discard the packaging for the plate. The ROI calibration plate can be used up to three times if it is stored in its original packaging sleeve.

Notes

4. (Standard plates only) Vortex the plate for 5 sec.

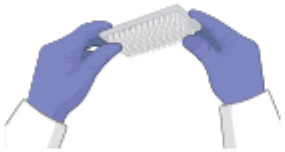
IMPORTANT! Do not vortex Fast plates.

(The remaining steps apply to both standard and Fast plates.)

5. Centrifuge the plate for 2 min at less than 1500 rpm.

IMPORTANT! The ROI calibration plate must be well mixed and centrifuged.

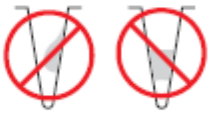
6. Verify that the liquid in each well of the ROI calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.



Correct

Liquid is at bottom of well.

Incorrect



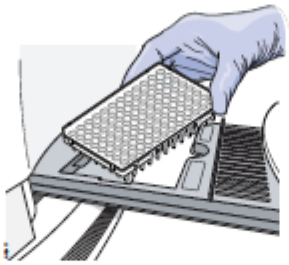
- Not centrifuged with enough force, *or*
- Not centrifuged for enough time

Notes

Load the Plate

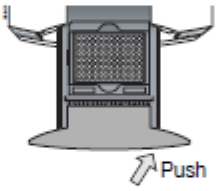
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.



Load standard plates (7500 system) with the notched A12 position at the top-right of the tray.	Load Fast plates (7500 Fast system) with the notched A1 position at the top-left of the tray.
	

3. Close the tray door. Apply pressure to the right side of the tray door at an angle.



Notes

Perform the Optical Calibration

Perform the Calibration

1. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the **Optical** tab.
3. In the Optical screen, click **Start Calibration.**
4. Complete the calibration as instructed by the wizard.

   The Optical Calibration dialog box displays four tabs:
   - **Overview** – Displays information describing the calibration.
   - **Setup** – Displays instructions for setting up the optical calibration. Clicking Next prompts opens the Run tab.
   - **Run** – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the calibration status (Passed/Failed).

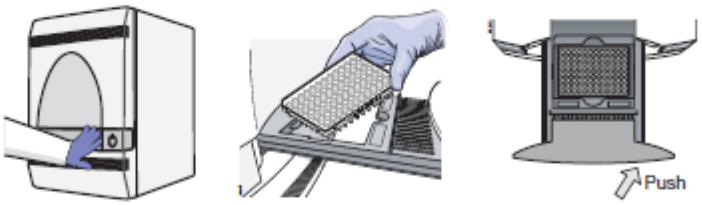
   If you cannot obtain a passing calibration, see "Troubleshoot the Background Calibration" on page 30.

Note: Before starting the calibration, the instrument may pause (up to 10 min) to allow the heated cover to reach temperature.

Unload the Plate

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Remove the calibration plate:
   a. Push the tray door to open it.
   b. Remove the calibration plate.
   c. Push the tray door to move it into the instrument.



Notes

2. Place the calibration plate inside its packaging sleeve. Return the packaged plate to the spectral calibration kit in the freezer.

IMPORTANT! Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it up to three times after you open it.

Continue with "Perform the Dye Calibration" on page 31.

Notes

Troubleshoot the Background Calibration

Problem/Symptom	Possible Cause	Action
Background Calibration Failed	One or more wells of the background plate produced spectra that exceed the maximum limit for the instrument.	1. Repeat the calibration using the same background plate. 2. If the calibration fails again, repeat the calibration using a different background plate. 3. If the calibration fails again, determine the source of the contamination, as explained in "How to Identify Contamination" below.

How to Identify Contamination

Signals that exceed the limit of normal background fluorescence may indicate fluorescent contaminants on the calibration plate or the sample block. Common contaminants include ink residue from permanent pens, powder from disposable gloves, and dust.

To determine the source and location of the contamination:

1. While viewing the raw spectra, locate the contaminated well position(s) by selecting successively smaller regions of the plate layout.
2. Rotate the background plate 180°, then perform the background calibration again.
3. Repeat step 1 to locate the contamination. If the well position(s) of the contamination in steps 1 and 3 are:
   - **Identical** – The sample block is contaminated. Decontaminate the sample block as explained in "Decontaminate the Sample Block" on page 59.
   - **Reversed** – The background plate is contaminated. Discard the background plate and perform the background run using a new background plate.

If the calibration fails after you replace the background plate and decontaminate the sample block:

1. Load a plate covered by a piece of black paper into the 7500/7500 Fast instrument.
2. Perform the background run as explained in this chapter.
3. After the run is complete, select all wells of the plate layout.
4. View the Spectral plot for the peak(s). If a peak is:
   - **Visible** – The optics of your 7500/7500 Fast instrument may be contaminated. Contact Applied Biosystems as explained in "How to Obtain Support" on page x.
   - **Absent** – The sample block is contaminated. Decontaminate the sample block as explained in "Decontaminate the Sample Block" on page 59.

Notes

4 Perform the Dye Calibration

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Overview

During a dye calibration, the Applied Biosystems 7500/7500 Fast Real-Time PCR System:

- Collects spectral data from a series of dye standards.
- Stores the spectral information for the dye standards in a pure spectra calibration file.

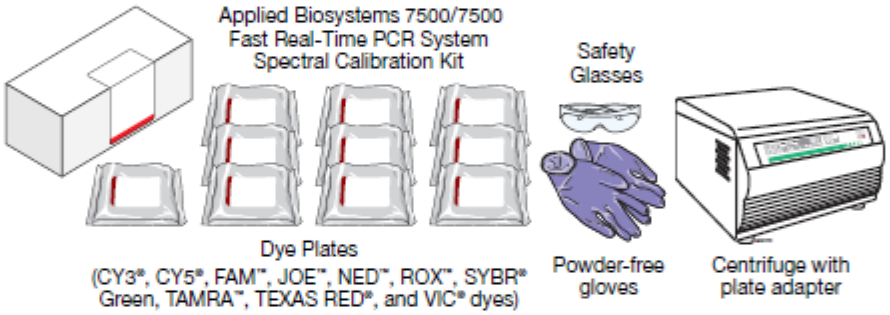
The software uses the pure spectra data during experiment runs to characterize and distinguish the individual contribution of each dye in the total fluorescence collected by the instrument. After each run, the 7500 software receives data in the form of a raw spectra signal for each reading. It determines the contribution of each fluorescent dye used in the sample by comparing the raw spectra to the pure spectra calibration data. When you save an experiment after analysis, the software stores the pure spectra with the collected fluorescence data for that experiment.

Note: The dye calibration is a user-performed maintenance procedure.

Time Required

1 hr

Materials Required



Note: If you store Applied Biosystems 7500/7500 Fast Real-Time PCR System dye plates in their original packaging in the freezer, you can use them to calibrate a 7500/7500 Fast instrument up to 3 times for 6 months after opening them.

When to Perform Dye Calibration

Perform a dye calibration:

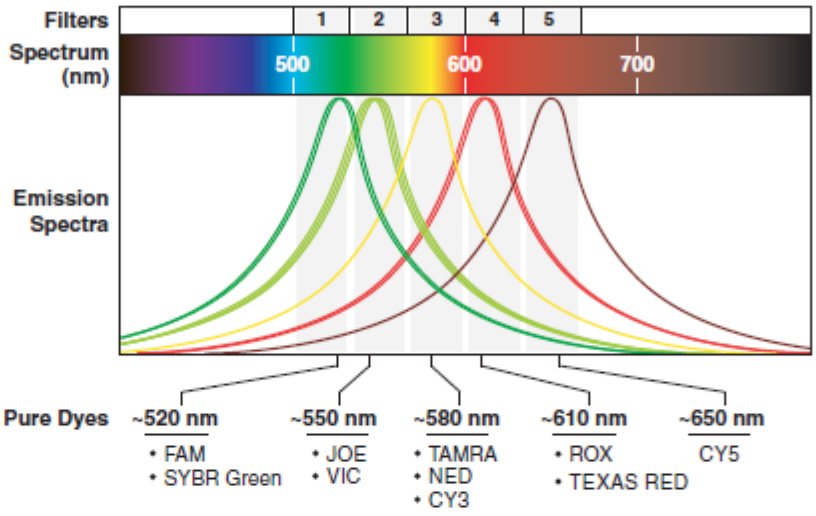
- When installing the 7500/7500 Fast system. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Every 6 months, or as often as necessary, depending on instrument use.

IMPORTANT! You must perform a background run before every series of dye calibrations. Because the age and use of instrument components can affect pure spectra readings, Applied Biosystems recommends performing a dye calibration at least every 6 months.

Notes

Dye Sets

The Applied Biosystems 7500/7500 Fast Real-Time PCR Systems use the following dye sets for calibration: CY3® dye, CY5® dye, FAM™ dye, JOE™ dye, NED™ dye, ROX™ dye, SYBR® Green dye, TAMRA™ dye, TEXAS RED® dye, and VIC® dye. The following figure shows the emission spectrum for each dye, and the filters and wavelengths at which each dye is read.



Custom Dye

The 7500/7500 Fast system can be used to run assays designed with custom dyes (dyes not supplied by Applied Biosystems). However, before using custom dyes with the 7500/7500 Fast instrument, you must create and run a custom calibration plate. The 7500 software uses the custom calibration plate to create a spectral standard to distinguish the custom dye in the fluorescence data collected during the run. See Appendix B for information on custom dye calibrations.

IMPORTANT! To use a custom dye on your 7500/7500 Fast system, it must fluoresce within the 520 to 650 nm spectral range measured by the 7500/7500 Fast instrument.

Notes

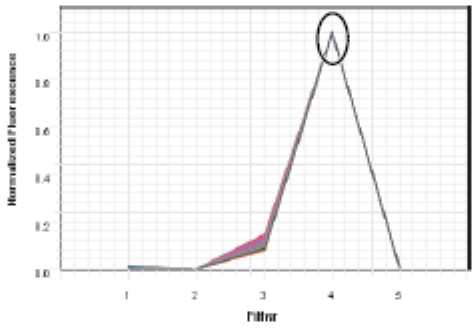
About the Analysis

The product of a dye calibration is a collection of spectral profiles that represent the fluorescence signature of each dye standard. Each profile consists of a set of spectra that correspond to the fluorescence collected from the wells of the spectral calibration plate. The 7500 software plots the resulting data for each spectral profile in a graph of fluorescence versus filter.

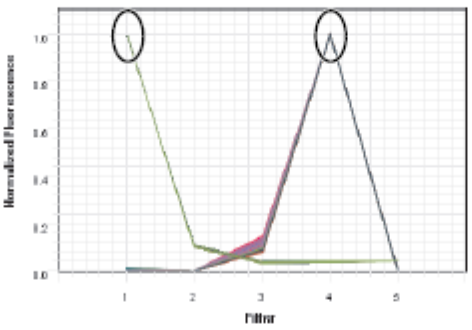
When the 7500 software extracts the calibration data from a dye run, it evaluates the fluorescence signal generated by each well in terms of the collective spectra for the entire calibration plate. Dye spectra are generally acceptable if they peak within the same filter as their group but diverge slightly at other wavelengths (see below).

The 7500 software can compensate for some differences in a spectral profile by replacing (auto-repairing) the spectra of unacceptable wells with the spectra of other wells on the reaction plate. However, the software allows only a few replacements and may reject the calibration if the spectra between neighboring wells vary significantly.

Note: Because the wells in a calibration plate contain dyes at identical concentrations, the resulting signals for the wells containing each dye should be similar. Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.



Acceptable Spectra
Spectra peak at the same wavelength and do not diverge significantly



Unacceptable Spectra
Spectra peak at the different wavelengths

Notes

Prepare the Dye Calibration Plates

IMPORTANT! Before performing a dye calibration, you must perform an ROI calibration (see page 8), a background calibration (see page 20), and an optical calibration (see page 25).

Prepare the Plates

IMPORTANT! Wear powder-free gloves when you handle the plate.

1. Obtain the spectral calibration kit from the freezer, then remove all of the dye plates.
2. Return the spectral calibration kit to the freezer.
3. Allow the dye plates to warm to room temperature (approximately 5 min).

IMPORTANT! Do not remove a dye plate from its packaging until you are ready to run it. The fluorescent dye in the wells of each dye plate is photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plate.

Note: If you store Applied Biosystems 7500/7500 Fast Real-Time PCR System dye plates in their original packaging in the freezer, you can use them to calibrate a 7500/7500 Fast instrument up to 3 times for 6 months after opening them.

Continue with "Perform the Dye Calibration" on page 36.

Notes

Perform the Dye Calibration

Perform the Calibration

1. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the **Dye** tab.
3. In the Dye screen, select **System Dye Calibration**.
4. Click **Start Calibration**.
5. Complete the calibration for each plate as instructed by the wizard.

IMPORTANT! The wizard guides you through the calibration of each dye separately. You must set up, run, and analyze each dye plate independently.

The Dye Calibration dialog box displays four tabs:

- **Overview** – Displays information describing the calibration.
  When the software prompts you to obtain the required materials, select the dyes that you want to calibrate.
- **Setup** – Displays instructions for setting up the dye calibration. Clicking Next prompts opens the Run tab.
  When the software prompts you to load each dye plate, prepare and load the plates as described in "Load a Dye Plate" on page 37.
- **Run** – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
- **Analysis** – Indicates the calibration status (Passed/Failed).

When the software prompts you to analyze the spectra collected from each dye plate, verify the status of the calibration:

- **Passed** – The 7500/7500 Fast instrument passed the calibration. Go to "Analyze the Calibration Data" on page 39.
- **Failed** – The 7500/7500 Fast instrument failed the calibration. Troubleshoot the error as described in "Troubleshoot the Dye Calibration" on page 43.

Note: Before starting the calibration, the instrument may pause (up to 10 min) to allow the heated cover to reach temperature.

Notes

Load a Dye Plate

Note: Because the wizard guides you through the calibration of each dye separately, perform the following procedure for each dye that you calibrate.

1. Remove the dye plate that is specified by the software from its packaging.

IMPORTANT! Do not discard the packaging for the plate. The plate can be used up to three times if it is stored in its original packaging sleeve.

2. (Standard plates only) Vortex the plate for 5 sec.

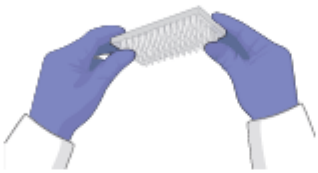
IMPORTANT! Do not vortex Fast plates.

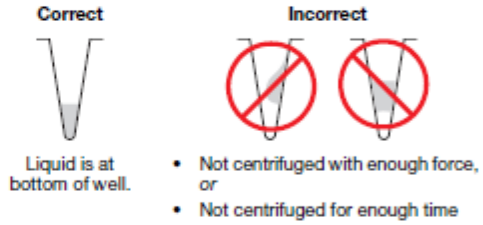
(The remaining steps apply to both standard and Fast plates.)

3. Centrifuge the plate for 2 min at less than 1500 rpm.

IMPORTANT! The plate must be well mixed and centrifuged.

4. Verify that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.



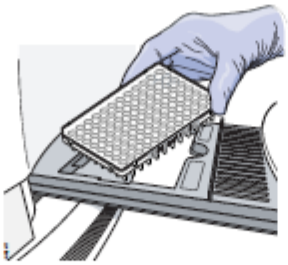


5. Verify that the dye plate that you are about to load matches the dye selected in the 7500 software.

6. Push the tray door to open it.

Notes

7. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.



Load standard plates (7500 system) with the notched A12 position at the top-right of the tray.	Load Fast plates (7500 Fast system) with the notched A1 position at the top-left of the tray.
	

8. Close the tray door. Apply pressure to the right side of the tray door at an angle.

Note: If you cannot open the tray, the sample block may be in its raised position, locking the tray position. To lower the block, select **Instrument ▸ Calibrate**, then exit the ROI Inspector.

Notes

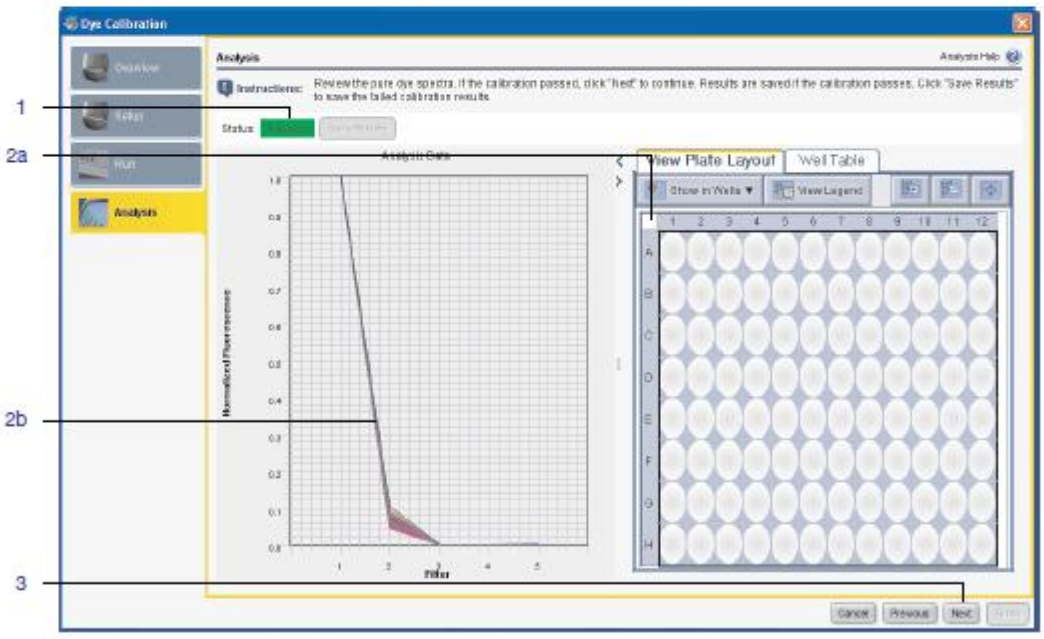
Analyze the Calibration Data

Note: Because the wizard guides you through the calibration of each dye separately, perform the following procedure for each dye that you calibrate.

1. Verify the status of the calibration:
   - **Passed** – The 7500/7500 Fast instrument passed the calibration. Go to step 2.
   - **Failed** – The 7500/7500 Fast instrument failed the calibration. Troubleshoot the error as described in "Troubleshoot the Dye Calibration" on page 43.
2. Verify the grouping of the dye spectra:
   a. In the plate layout, select the wells of the plate.
   b. Inspect the raw data. For each spectrum, verify that the peak is:
      - Within the detectable range for the 7500/7500 Fast instrument.
      - Free of irregular spectral peaks.
      - Present in the correct channel for the dye (see Table 1 on page 41).

   If a spectrum does not match the criteria above, troubleshoot the problem as described in "Troubleshoot the Dye Calibration" on page 43.

   Note: Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.
3. If all spectra are acceptable, then click **Next**.



Notes

4. Remove the calibration plate:

WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

a. Push the tray door to open it.

b. Remove the calibration plate.

c. Push the tray door to close it.

d. Place the calibration plate inside its packaging sleeve. Return the packaged plate to the spectral calibration kit in the freezer.

Note: If you store Applied Biosystems 7500/7500 Fast Real-Time PCR System dye plates in their original packaging in the freezer, you can use them to calibrate 7500/7500 Fast instruments up to 3 times for 6 months after opening them.

5. After you remove the dye plate as instructed, click **Finish**.

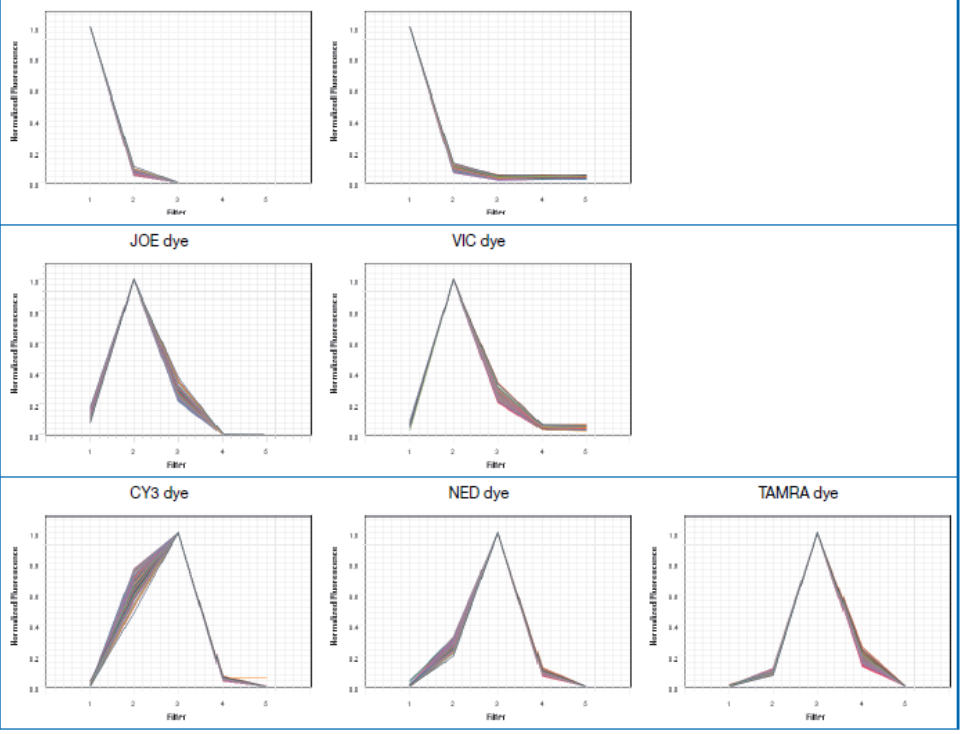
6. Prepare and run the next plate as explained in "Prepare the Plates" on page 35.

Notes

Notes

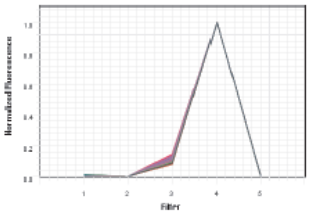
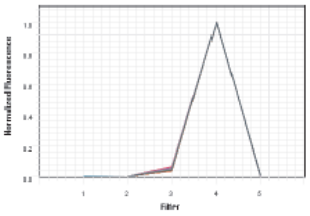
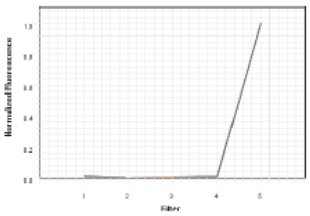
Table 1 7500/7500 Fast system dye spectra

Filter	Peak (nm)	Dye/Spectra
1	~520	FAM dye, SYBR Green dye
2	~550	JOE dye, VIC dye
3	~580	CY3 dye, NED dye, TAMRA dye



Notes

Table 1 7500/7500 Fast system dye spectra

Filter	Peak (nm)	Dye/Spectra
4	~610	ROX dye  TEXAS RED dye 
5	~670	CY5 dye 

Troubleshoot the Dye Calibration

Problem/Symptom	Possible Cause	Action
One or more raw spectra are at or below the detectable threshold for the calibration.	• The spectral calibration plate was centrifuged insufficiently. • The spectral calibration plate contains old or insufficient reagents. • If you are running a custom spectral calibration plate, the dye may not be present at a sufficient concentration.	1. Unload the 7500/7500 Fast instrument and view the wells of the spectral calibration plate. If the liquid in the wells is not: – At the bottom of the wells, centrifuge the plate for a longer time, then repeat the calibration. – Equivalent in volume, the plate is not sealed and the reagents have evaporated. Discard it and run another. 2. If the spectral calibration plate appears to be normal, discard the plate and run another. 3. If the problem persists, contact Applied Biosystems as explained in "How to Obtain Support" on page x. **Note:** If you are running a custom spectral calibration plate, create another plate but increase the concentration of the dye that produced insufficient signal.
One or more raw spectra exceed the maximum limit for the 7500/7500 Fast instrument.	• Fluorescent contaminants are on the sample block(s) or spectral calibration plate. • If you are running a custom spectral calibration plate, the dye may be too concentrated.	Verify that contaminants are not present by performing a background calibration as explained in Chapter 3, "Perform the Background Calibration and Optical Calibration." If the background calibration does not show sample block contamination, the spectral calibration plate may be contaminated. **Note:** If you are running a custom spectral calibration plate, create another plate but decrease the concentration of the dye that exceeds the detectable limit.
The spectra contain peaks in more than one filter.	Fluorescent contaminants are on the sample block(s) or spectral calibration plate.	

Notes

Notes

5 Verify the Instrument Performance

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking ? in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Overview

Perform the TaqMan® RNase P Instrument Verification Plate run to verify the performance of an Applied Biosystems 7500/7500 Fast Real-Time PCR System.

Time Required 1 hr

Materials Required

When to Perform the RNase P Experiment

Applied Biosystems recommends performing an RNase P experiment:

- When installing the 7500/7500 Fast system. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- After moving the instrument to another location.
- As needed to verify the function of the 7500/7500 Fast instrument.

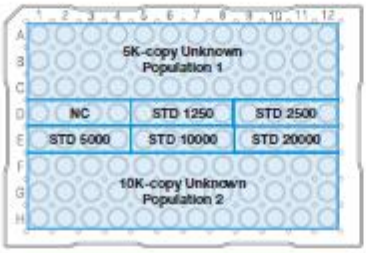
About the RNase P Plate

The RNase P plate is preloaded with the reagents necessary for the detection and quantitation of genomic copies of the human RNase P gene (a single-copy gene encoding the RNase moiety of the RNase P enzyme).

Each well contains:

- 1X TaqMan® Fast Universal PCR Master Mix, No AmpErase® UNG
- RNase P primers
- FAM™ dye-labeled probe
- Known concentration of human genomic DNA template

The figure below illustrates the arrangement of the standard and unknown populations on the RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10,000, and 20,000 copies), two unknown populations (5000 and 10,000 copies), and negative control wells (NC).



Notes

After the run, the 7500 software:

1. Generates a standard curve from the averaged threshold cycle (C_T) values of the replicate groups of standards.
2. Calculates the concentration of the two unknown populations using the standard curve.
3. Calculates the following to assess the 7500/7500 Fast instrument performance:

$$[(CopyUnk_2) - 3(\sigma_{CopyUnk2})] > [(CopyUnk_1) + 3(\sigma_{CopyUnk1})]$$

where:

- $CopyUnk_1$ = Average copy number of unknown #1 (5,000-copy population)
- $\sigma_{CopyUnk1}$ = Standard deviation of unknown #1 (5,000-copy population)
- $CopyUnk_2$ = Average copy number of unknown #2 (10,000-copy population)
- $\sigma_{CopyUnk2}$ = Standard deviation of unknown #2 (10,000-copy population)

Installation Specification

The 7500/7500 Fast instrument passes the installation specification if the inequality holds and the instrument successfully distinguishes between 5,000 and 10,000 copies with a statistical confidence level of 99.7%.

To meet the installation specification, you can omit a limited number of outlier wells from the 5,000- and 10,000-copy unknown populations.

Instrument	Maximum Number of Outlier Wells That Can Be Removed			
	Unknown Population		Standard (STD)	Negative Controls (NC)
	5,000-copy	10,000-copy		
7500 System	6	6	0	0
7500 Fast System				

Notes

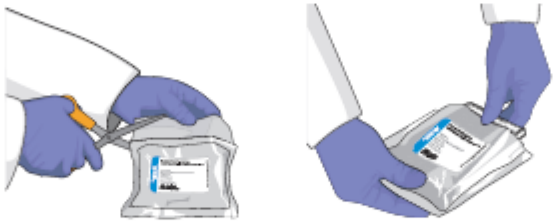
Set Up the Experiment

Prepare the TaqMan® RNase P Fast Instrument Verification Plate for the run.

Prepare the RNase P Plate

IMPORTANT! Wear powder-free gloves when you handle the RNase P plate.

1. Obtain the TaqMan® RNase P Fast Instrument Verification Plate from the freezer, then allow the reaction plate to warm to room temperature (for approximately 5 min).
2. Remove the RNase P plate from its packaging.



3. (Standard plates only) Vortex the plate for 5 sec.

IMPORTANT! Do not vortex Fast plates.

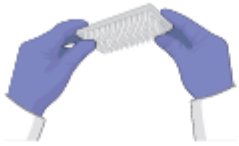
(The remaining steps apply to both standard and Fast plates.)

4. Centrifuge the reaction plate for 2 min at less than 1500 rpm.

IMPORTANT! The reaction plate must be well mixed and centrifuged.

5. Verify that the liquid is at the bottom of each well of the reaction plate. If not, centrifuge the reaction plate again at a greater rpm and for a longer time.

IMPORTANT! Do not allow the bottom of the RNase P plate to become dirty. Fluids and other contaminants that adhere to the bottom of the reaction plate can contaminate the sample block(s) and cause an abnormally high background signal.



Correct	Incorrect
 Liquid is at the bottom of the well.	 • Not centrifuged with enough force, or • Not centrifuged for enough time

Notes

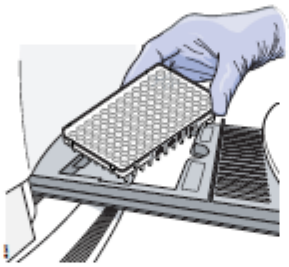
Run the Experiment

After preparing the TaqMan® RNase P Fast Instrument Verification Plate, load the plate into the 7500/7500 Fast instrument and start the run.

Load the Plate

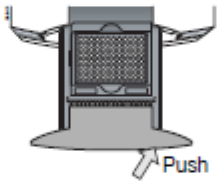
WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.



Load standard plates (7500 system) with the notched A12 position at the top-right of the tray.	Load Fast plates (7500 Fast system) with the notched A1 position at the top-left of the tray.
	

3. Close the tray door. Apply pressure to the right side of the tray door at an angle.



5

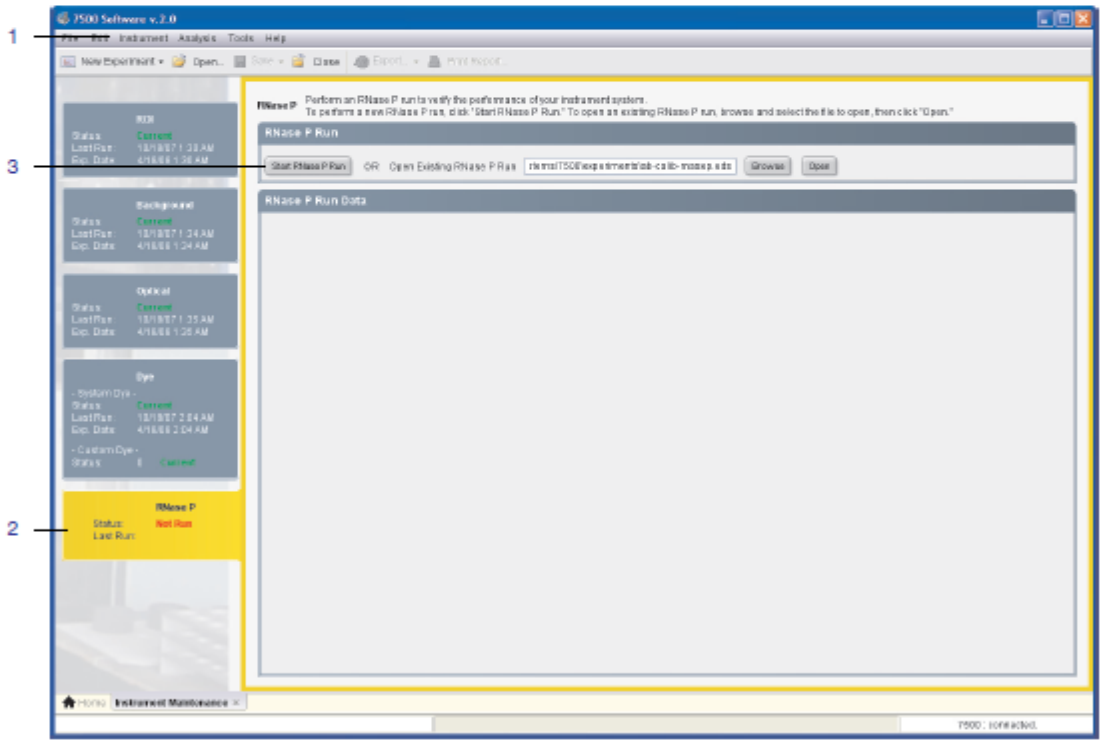
Notes

Start the Run

1. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the **RNase P** tab.
3. In the RNase P screen, click **Start RNase P Run.**
4. Complete the calibration as instructed by the wizard.

   The RNase P dialog box displays four tabs:
   - **Overview** – Displays information describing the calibration.
   - **Setup** – Displays instructions for setting up the RNase P run. Clicking Next prompts opens the Run tab.
   - **Run** – Clicking START RUN starts the run process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the run status (Passed/Failed).

Note: Before starting the run, the instrument may pause (up to 10 min) to allow the heated cover to reach the correct temperature.



Notes

Analyze the Experiment

Review the data to verify the results of the experiment.

Verify the Results of the Analysis

Note: After the 7500 software completes the RNase P run, it automatically analyzes the run and displays the results in the Analysis screen.

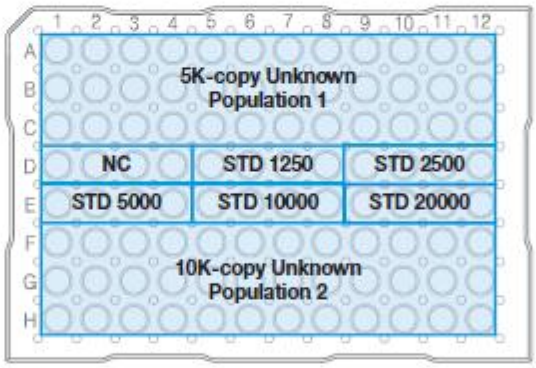
1. In the Analysis screen of the RNase P Run wizard, verify the status of the run:
   - **Passed** – The 7500/7500 Fast instrument passed the RNase P run. Go to step 5 on page 53.
   - **Failed** – The 7500/7500 Fast instrument failed the RNase P run. Go to step 2 to review the data for outliers.

   If the run fails, the automated analysis may have included outliers that caused the initial analysis to fail. Experimental error may cause some wells to be amplified insufficiently or not at all. These wells typically produce C_T values that differ significantly from the average for the associated replicate wells. If included in the calculations, these outlying data (outliers) can result in erroneous measurements.

2. In the Amplification Plot, select **Ct vs. Well** from the Plot Type drop-down list.

3. Verify the uniformity of each replicate population on the reaction plate (controls, standards, and unknowns) by comparing the groupings of C_T values:

   a. In the plate layout, select the wells containing the 10,000-copy unknown population (wells rows F, G, and H).



   b. In the plot, verify that the C_Ts of the replicate population are equivalent.

   Note: The numbers on the X-axis of the plot correspond to the wells of the reaction plate. Beginning with well A1, the wells are numbered from left-to-right, and top-to-bottom.

Notes

c. If an outlier is in the selected population, select the corresponding well in the plate layout, then click **Omit** to remove the well from the analysis.

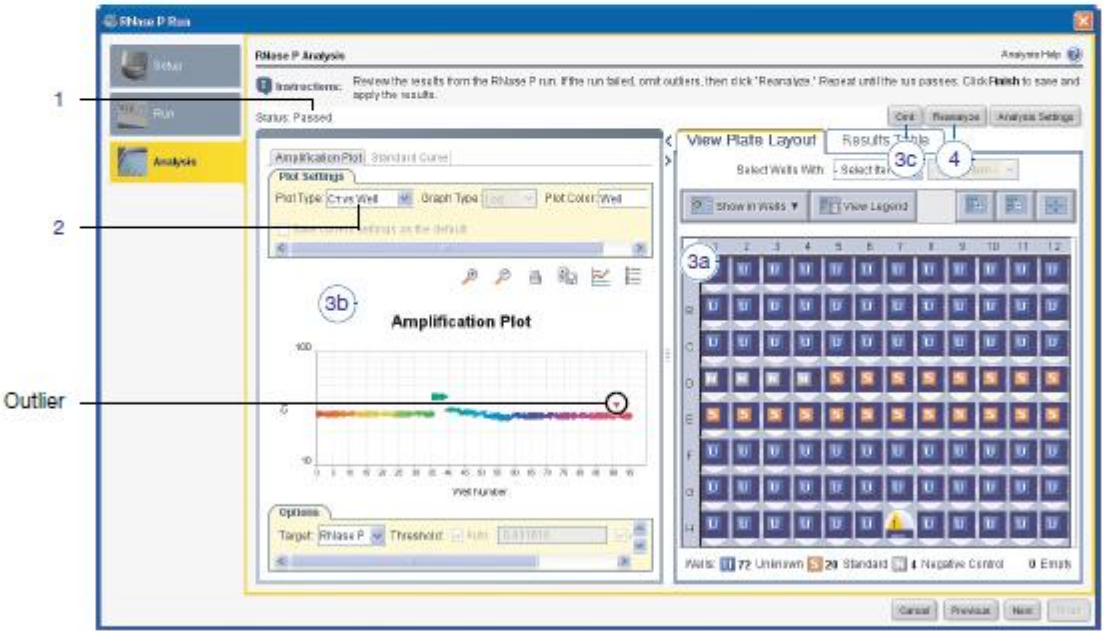
Instrument	Maximum Number of Outlier Wells That Can Be Removed			
	Unknown Population		Standard (STD)	Negative Control (NC)
	5,000-copy	10,000-copy		
7500 System	6	6	0	0
7500 Fast System				

IMPORTANT! If the number of outliers exceeds the limit in the table above, order another RNase P plate and repeat the experiment.

d. Repeat steps 3a through 3c for each replicate population (unknowns, standards, and negative controls) on the reaction plate.

4. Click **Reanalyze** to analyze the run without the outliers.

If the status of the RNase P Run is "**Failed**" after performing steps 2 through 4, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Applied Biosystems as explained in "How to Obtain Support" on page x.



Notes

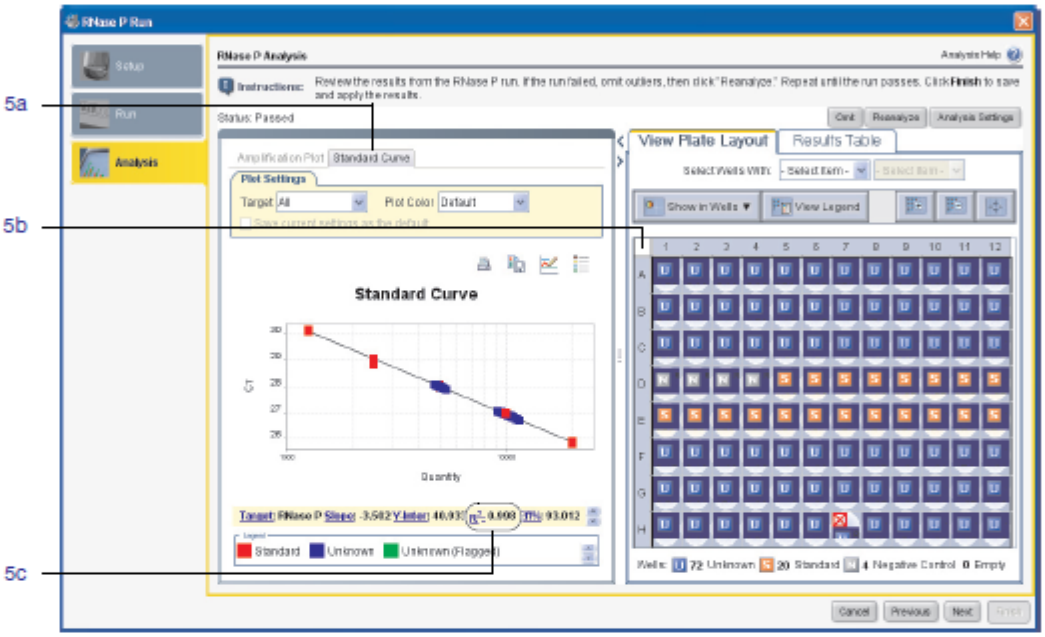
5. Review the standard curve:

   a. Select the **Standard Curve** tab.

   b. Click the upper-left corner of the Plate Layout to select all wells.

   c. Verify that the R2 value is greater than or equal to 0.990.

   If the R2 value is less than 0.990, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Applied Biosystems as explained in "How to Obtain Support" on page x.



6. Click **Next**, then remove the calibration plate.

   WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

   a. Push the tray door to open it.

   b. Remove the calibration plate.

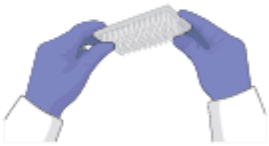
   c. Push the tray door to move it into the instrument.

7. Discard the plate.

8. Click **Finish**, then click **Yes** when prompted to save the experiment.

Notes

Troubleshoot the RNase P Experiment

Problem/Symptom	Possible Cause	Action
More than the maximum number of outliers are present in RNase P data	• Possible contamination • Pipetting inaccuracy	Contact your Applied Biosystems service and sales representative to order a replacement TaqMan® RNase P Instrument Verification Plate. If the replacement RNase P plate fails, contact Applied Biosystems technical support or your service representative for further assistance.
The RNase P plate verification run failed	• Insufficient centrifugation • Defective plate seal	**WARNING** **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature. 1. Unload the RNase P plate from the instrument: a. Push the tray door to open it. b. Remove the RNase P plate from the tray. c. Push the tray back into the instrument.   2. Hold the plate up to a light source, and verify that all wells contain the same volume of fluid. If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation. Also, compare the position of the wells that have lower volumes with the outliers that you have removed from the plate. If the well positions coincide, the heat seal on the plate may be defective and resulted in the evaporation of the associated samples. 3. Contact your Applied Biosystems service and sales representative to order a replacement TaqMan® RNase P Instrument Verification Plate. If the replacement RNase P plate fails, contact Applied Biosystems technical support or your service representative for further assistance.

Notes

6 User-Performed Maintenance

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Monitor the 7500/7500 Fast System

You can monitor the state of the Applied Biosystems 7500/7500 Fast Real-Time PCR System using the Function Test, Lamp Status/Replacement, and Instrument Log tools of the 7500 software. The tools enable you to assess the health of the 7500/7500 Fast system, check the replacement status of the instrument lamp, and view a recent history of instrument activity.

View the Instrument Log

Use the Instrument Log to view the recent event history of the 7500/7500 Fast system. The log displays the major instrument activity for either the most recent 25 runs (including calibrations), or the events that pertain only to a specific EDS file.

Display the Instrument Log

1. In the 7500 software, select **Instrument ▸ Instrument Events Log**.
2. In the Instrument Events Log dialog box, select either:
   - **System Log** – To view events that occurred during the 25 most recent runs (experiments) or calibrations.
   - **Document Log** – To view events that pertain only to the experiment currently open in the 7500 software.
3. If necessary, modify the data displayed by the table by filtering the data and adding or removing columns.

To...	Action
Filter the data in the events table	1. In the Filter drop-down list, select a property. 2. Enter the appropriate conditions into the drop-down lists and fields that appear automatically. 3. Click **Apply** to filter the data. **Note:** To reset the log, select **Filter ▸ Show All Records**.
Add or remove columns to/from the events table	Click **Show Columns**, then select the column desired column in the drop-down list.
Sort the data in the events table	Click the heading of the column of interest once to sort the data in ascending order. Click the column heading again to sort the data in reverse order.
Export the contents of the instrument event log	1. Select the rows of interest in the event table, 2. Press **Ctrl+C** to copy the data. 3. Paste the data into a spreadsheet application or a text file. **Note:** The software exports the data in tab-delimited format.

4. When you finish viewing the events, click the close box to close the dialog box.

Notes

Monitor the Instrument Status

Use the Function Test dialog box to perform a high-level diagnostic of the major 7500/7500 Fast system components. In general, you need not perform the function tests unless you experience a suspected hardware failure, or you are instructed to do so by an Applied Biosystems representative.

Perform Function Tests of System Components

1. In the 7500 software, select **Instrument** ▸ **Function-Test**.
2. Perform function tests as needed.

   To test:

   - All system components – Click **All Tests**, then wait for the software to perform all of the function tests.
   - One or more specific components – Click one or more of the following.

     USB – Tests the universal serial bus (USB) connection between the 7500/7500 Fast instrument and computer. The test passes if the 7500 software can establish communication with the 7500/7500 Fast instrument.

     CCD – Tests the CCD camera in the 7500/7500 Fast instrument. The test passes if the camera can capture an image.

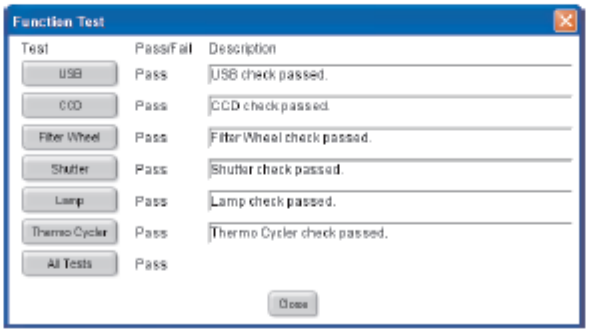
     Filter Wheel – Tests the filter wheel in the 7500/7500 Fast instrument. The test passes if the filter wheel controller is running the correct version of firmware.

     Shutter – Tests the optic shutter of the 7500/7500 Fast instrument. The test passes if the shutter controller is running the correct version of firmware.

     Lamp – Tests the halogen lamp of the 7500/7500 Fast instrument. The test passes if the lamp controller is running the correct version of firmware.

     Thermal Cycler – Tests the thermal cycler sample block in the 7500/7500 Fast instrument. The test passes if the thermal cycler controller is running the correct version of firmware.

   When the 7500 software completes a test, the software reports the pass/fail status of the test and provides a description of the outcome.



3. When you finish testing, click **Close**.

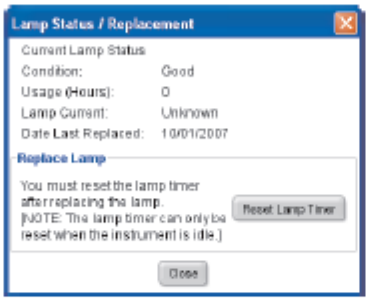
Notes

Monitor the Lamp Status

Use the Lamp Status/Replacement dialog box to monitor the status of the halogen lamp that the 7500/7500 Fast instrument uses to illuminate samples during runs.

Check the Lamp Status

In the 7500 software, select **Instrument ▸ Lamp Status/Replacement** to determine the status of the halogen lamp.



The Lamp Status/Replacement dialog box displays:

- **Condition** – Indicates one of the following conditions:
  - **Good** – The lamp is functioning well and does not need to be replaced. Click **Close**.
  - **Failed** – The lamp bulb must be replaced. Click **Close**, then replace the lamp as explained in "Replace the Halogen Lamp" on page 63.
  - **Change Soon** – The lamp usage is above 2000 hrs; Applied Biosystems recommends that you replace it. Click **Close**, then decide whether or not to replace the lamp. If you choose to replace the lamp, see "Replace the Halogen Lamp" on page 63.
- **Usage (Hours)** – The total number of hours that the lamp has been illuminated.
- **Lamp Current** – The output current of the lamp in amperes (A). Low current can indicate a potential future failure of the lamp.
- **Date Last Replaced** – The date of the last lamp replacement.

Warnings

The 7500 software can display the following warnings before or during a run:

Message	Description
Warning – Cannot detect sufficient current from lamp. Either lamp is not installed properly or needs to be replaced.	The lamp current is below the acceptable level at the start of the run. You cannot proceed with the run until you replace the halogen bulb as explained in "Replace the Halogen Lamp" on page 63.
Warning – Cannot detect sufficient current from lamp. Either lamp is not installed properly or needs to be replaced.	The 7500 software stopped the run because the lamp current decreased below the acceptable level during the run. You cannot proceed with the run until you replace the halogen bulb as explained in "Replace the Halogen Lamp" on page 63. Click **OK** in the message box, inspect the Instrument Log, then replace the lamp bulb.
Warning - The lamp usage has exceeded 2000 hr. We recommend replacing the lamp soon to ensure optimal assay performance.	The lamp usage exceeds 2000 hr at the start of a run. Click **Cancel Run**, then replace the lamp, or click **Continue Run**. Rerun ROI, Background, Optical and Dye calibrations.

Notes

Decontaminate the Sample Block

Perform the following procedure to eliminate fluorescent contaminants from the sample block of the 7500/7500 Fast instrument. Fluorescent contamination is generally evident in failed background runs where one or more wells consistently exhibit abnormally high signals.

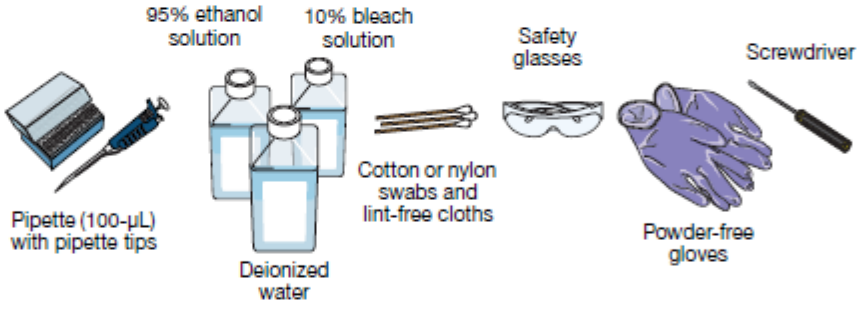
CAUTION **PHYSICAL INJURY HAZARD.** Do not remove the instrument cover. There are no components inside the 7500/7500 Fast system that you can safely service yourself. If you suspect a problem, contact an Applied Biosystems Service Representative.

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

CAUTION Before using a cleaning or decontamination method other than those recommended by the Applied Biosystems, verify with Applied Biosystems that the proposed method will not damage the equipment.

Time Required 30 min

Materials Required



Clean the Sample Block

IMPORTANT! Wear powder-free gloves when you perform this procedure.

1. Identify the contaminated wells of the sample block (see "How to Identify Contamination" on page 30).
2. Remove the plate and the tray holder from the 7500/7500 Fast instrument:
   a. Push the tray door to open it.
   b. Remove the plate and the tray holder.

Notes

c. Close the tray door. Apply pressure to the right side of the tray door at an angle.

3. Manually raise the block:

   a. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.

   b. In the ROI tab of the Instrument Maintenance Manager, click **Start Manual Calibration.**

   c. In the ROI Inspector dialog box, click **Move Block**.

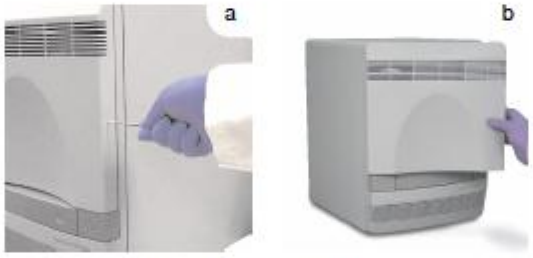
   d. When the ROI Inspector dialog box displays "Block Down," click **Done**.

4. Power off, then unplug the 7500/7500 Fast instrument. Allow it to cool for 15 min.

5. Open the access door to the 7500/7500 Fast instrument.

   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.

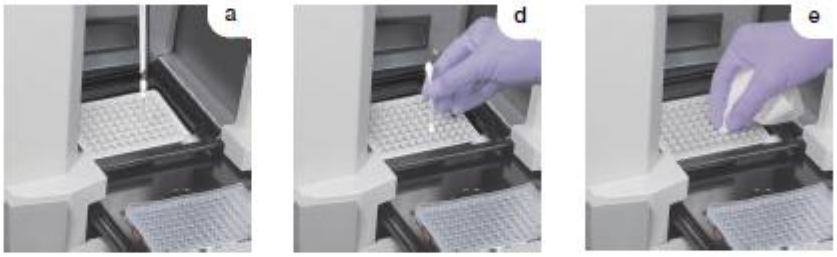
   b. Open the access door.



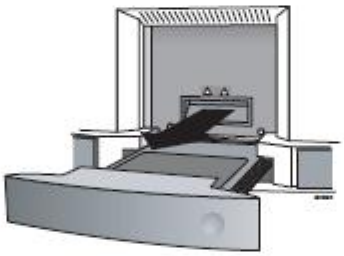
6. Lift the latch, then push the heated cover door to the back of the instrument.

Notes

7. Clean the contaminated wells of the sample block using a small volume of deionized water:
   a. Pipette a small volume of deionized water into each contaminated well.
   b. Pipette the water up and down several times to rinse the well.
   c. Pipette the water to a waste beaker.
   d. Using a cotton swab, scrub inside of each contaminated well.
   e. Using a lint-free cloth, absorb the excess deionized water.



8. Pull the heated cover door to the front of the 7500/7500 Fast instrument. Lift the latch, then secure the heated cover door to the cross bar.



9. Close the access door to the 7500/7500 Fast instrument.

10. Plug in, then power on the 7500/7500 Fast system.

11. Verify that you have eliminated the contamination by performing a background calibration run (see "Perform the Background Calibration" on page 20).

Notes

12. If the contamination remains, repeat steps 1 through 6, then clean the contaminated wells of the sample block using 95% ethanol solution:
    a. Pipette a small volume of 95% ethanol solution into each contaminated well.
    b. In each contaminated well, pipette the solution up and down several times to rinse the well.
    c. Pipette the ethanol solution to a waste beaker.

IMPORTANT! Always use deionized water to rinse wells after cleaning with bleach or ethanol solution.

13. Repeat steps 7 through 11 to rinse the wells of the sample block and to verify that you have eliminated the contamination.
14. If the contamination remains, repeat steps 1 through 6, then clean the contaminated wells of the sample block using 10% bleach solution:
    a. Pipette a small volume of 10% bleach solution into each contaminated well.
    b. In each contaminated well, pipette the solution up and down several times to rinse the well.
    c. Pipette the bleach solution to a waste beaker.

IMPORTANT! Always use deionized water to rinse wells after cleaning with bleach or ethanol solution.

15. Repeat steps 7 through 11 to rinse the wells of the sample block and to verify that you have eliminated the contamination.

    If the contamination remains, contact Applied Biosystems support (see "How to Obtain Support" on page x).
16. Ensure that the heated cover door is completely closed and latched. If it is not, the 7500 software displays an error message.

Notes

Replace the Halogen Lamp

Replace the halogen lamp after approximately 2000 hr of life.

WARNING **PHYSICAL INJURY HAZARD.** The 7500/7500 Fast system and lamp are hot! The lamp can become very hot while in use. Allow the lamp to cool for 15 min and put on protective, powder-free gloves before handling it.

CAUTION **PHYSICAL INJURY HAZARD.** Wear disposable, powder-free gloves when handling the lamp to prevent burns and to prevent shortening the life of the replacement lamp.

WARNING **WARNING.** This instrument is designed for 12 V, 75 W halogen lamps only.

Time Required 30 min

Materials Required

Halogen bulb (12 V, 75 W) | Screwdriver, small | Powder-free gloves | Safety glasses

Replace the Lamp

IMPORTANT! Wear powder-free gloves when you handle the lamp.

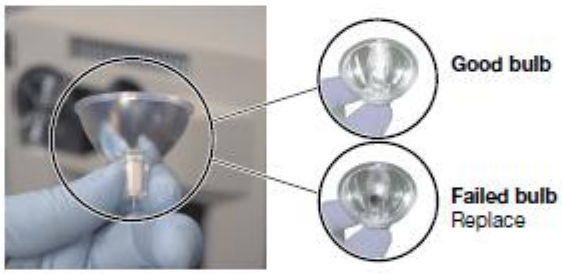
1. Power off, then unplug the 7500/7500 Fast system. Allow the instrument to cool for 15 min.
2. Open the access door to the 7500/7500 Fast system:
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
   b. Open the access door.

Notes

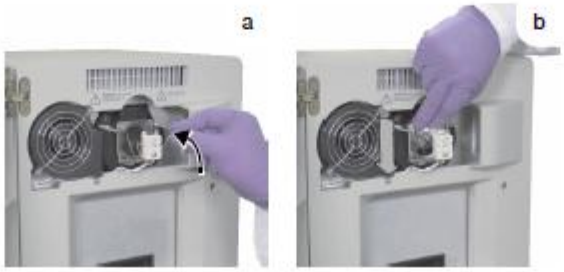
3. Remove the lamp from the instrument:
   a. Slide the lamp release lever downward.
   b. Firmly grasp the lamp and lift it up and out of the slotted mount.

IMPORTANT! Do not touch the lamp without powder-free gloves. Finger prints shorten the lamp life.

4. Inspect the lamp for signs of failure (carbon typically coats the inside of failed lamps).



5. Install the new lamp into the instrument:
   a. Slide the lamp release lever upward.
   b. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.



Notes

6. Close the access door.

7. Plug in and power on the 7500/7500 Fast system.
8. Open the ROI Inspector dialog box:
   a. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
   b. In the ROI tab of the Instrument Maintenance Manager, click **Start Manual Calibration.**
9. In the ROI Inspector dialog box, select **Lamp Control ▸ Idle**.
10. While the instrument is running, look through grating of the access door and verify that the lamp is illuminated, then click **Done**.

11. If the lamp is illuminated, select **Instrument ▸ Lamp Status/Replacement** in the 7500 software, click **Reset Lamp Timer**, then click **OK**.

    If the lamp is not illuminated, the replacement halogen lamp may be defective. Replace the lamp again. If the second lamp does not illuminate, check the instrument fuses for failure (see page 66).
12. Perform the following calibrations after replacing the lamp. See:
    - Chapter 2, Perform the Regions of Interest (ROI) Calibration
    - Chapter 3, Perform the Background Calibration and Optical Calibration
    - Chapter 4, Perform the Dye Calibration
    - Chapter 5, Verify the Instrument Performance

Notes

Replace the Instrument Fuses

Replace the 7500/7500 Fast instrument fuses when the fuses fail.

CAUTION **FIRE HAZARD.** For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the instrument.

Time Required 30 min

Materials Required

Fuses (2), 12.5 A, 250 V, 5 x 20 mm | Flathead screwdriver | Powder-free gloves | Safety glasses

Replace the Fuses

1. Power off the instrument, then unplug it.
2. Using a flat-head screwdriver, unscrew and remove the fuse holders from the instrument.

3. Remove each fuse from its fuse holder and inspect it for damage. Carbon typically coats the inside of failed fuses.

Notes

4. Replace each failed fuse with a 12.5 A, 250 V, 5 × 20-mm fuse.

Note: The voltage and amperage ratings are on the fuse holder.

5. Install the fuse holder.

6. Plug in, then power on the instrument.

   The installation is successful if the instrument powers on.

Note: Fuse failure can result from fluctuations in the supplied power to the instrument. To prevent further failures, consider installing an electrical protective device, such as a UPS or other surge protector. For more information about fuses, see the *7500/7500 Fast Site Preparation Guide*.

Update the Windows Operating System

Do not upgrade or update the Microsoft Windows® operating system of the computer running the 7500 software without first consulting the software release notes or the Applied Biosystems website. Future versions of the Windows® operating system and updates to the operating system can conflict with the 7500 software.

Determine Compatibility of an Upgrade or Update

1. Open **D:\Applied Biosystems\7500 Software v2.0**, double-click **release-notes.html**, then read the *7500 Software Release Notes* for the compatibility of interest.
2. If the release notes do not mention the compatibility, use an internet browser to visit **www.appliedbiosystems.com**, then search the website for the compatibility of interest.
3. If the website does not contain the information of interest, contact Applied Biosystems (see "How to Obtain Support" on page x).

Notes

Update the 7500 Software

If you want to update the 7500 software, prepare your computer by exporting the application libraries and backing up your experiment files.

Visit the Applied Biosystems Website

You can obtain 7500 software updates directly from the service section of the Applied Biosystems website. For the latest services and support information for the 7500/7500 Fast instrument:

1. Go to **https://www2.appliedbiosystems.com/support/software/**
2. In the Software Downloads page, select the appropriate instrument from the drop-down list.
3. In the Software Downloads page for your instrument, click **Updates - Patches**.

The website opens the page describing the latest software updates for the 7500 software.

Prepare for the Upgrade

Before updating the 7500 software:

1. Back up the application libraries:
   a. In the main menu of the 7500 software, select **Tools** ▸ *<desired library>*.
   b. When the library dialog box opens, select the element(s) that you want to export, then click **Export**.
   c. In the Export dialog box, click **Save** to archive the selected records.
   d. Repeat steps 1a through 1c for the remaining libraries to archive them.
2. Back up all experiment files by creating a copy of the directory that you are using to store files.

   The default directory for experiments is:

   D:\Applied Biosystems\7500\experiments

Notes

Store, Move, and Install the 7500/7500 Fast System

This chapter covers:

Note: For more information about any of the topics discussed in this guide, access the Help from within Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ 7500 Software Help**.

Notes

Store the 7500/7500 Fast System

The Applied Biosystems 7500/7500 Fast Real-Time PCR System can be powered off and stored for extended periods of time. The length of the period of inactivity determines the method you use to power off the instrument.

Time Required 5 min

Materials Required ABI PRISM® Optical 96-Well Reaction Plate or Optical 96-Well Fast Plate (unused)

Prepare the Instrument

1. Open the instrument tray door.
2. If the tray contains a plate, remove it.
3. If you plan to store the 7500/7500 Fast instrument for more than a week or you plan to move the instrument, load an unused plate into the tray.

   Note: The empty plate protects the internal components of the 7500/7500 Fast instrument during transport or during periods of inactivity lasting more than a week.

4. Push the tray door to move it into the instrument.

5. Press the instrument power button.
6. Power off the computer and monitor:
   a. Select **Start** ▸ **Shut Down**.
   b. In the Shut Down Windows dialog box, select **Shut Down**.
   c. Power off the monitor.

Notes

Move the 7500/7500 Fast System

Perform this procedure to safely move the 7500/7500 Fast instrument short distances (for example, between laboratories of the same building).

CAUTION **PHYSICAL INJURY HAZARD.** Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least 2 people are required to lift the 7500/7500 Fast system.

IMPORTANT! Moving your Applied Biosystems 7500/7500 Fast Real-Time PCR System can create subtle changes in the alignment of the instrument optics.

Materials Required

ABI PRISM® Optical 96-Well Reaction Plate and Optical 96-Well Fast Plate (unused)

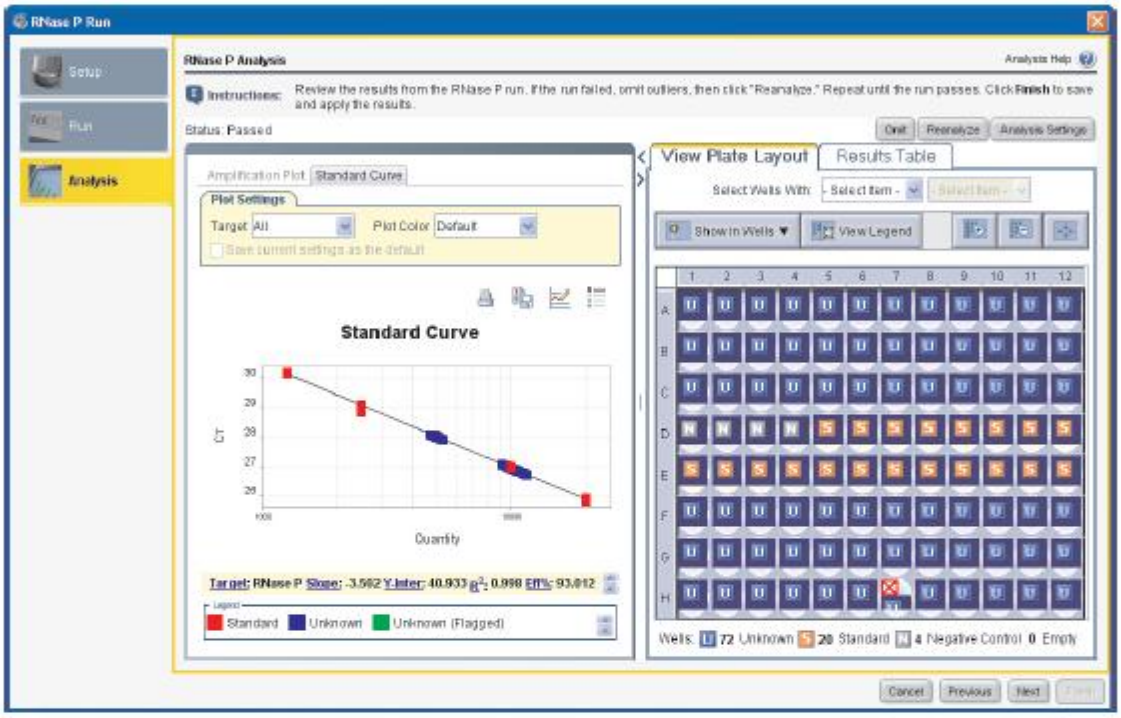
Prepare the Instrument

1. Load the empty reaction plate into the 7500/7500 Fast instrument.
2. Using the ROI Inspector, manually raise the sample block:
   a. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
   b. In the ROI tab of the Instrument Maintenance Manager, click **Start Manual Calibration.**
   c. In the ROI Inspector dialog box, click **Block Up**.
3. Power off the 7500/7500 Fast instrument and computer.
4. Disconnect all 7500/7500 Fast system components.
5. Move the 7500/7500 Fast system according to the following guidelines:

   - Verify that the surface on which you will place the instrument can support at least 54.5 kg (120 lbs).
   - Verify that the pathway to the final position of the instrument is clear of obstructions.
   - Keep your spine in a good neutral position.
   - Bend at the knees and lift with your legs.
   - Do not lift an object and twist your torso at the same time.
   - Coordinate your intentions with your assistant before lifting and carrying.

Notes

6. Reconnect the components of the 7500/7500 Fast system (see "Set Up the 7500/7500 Fast System" on page 73).
7. Run a TaqMan® RNase P Instrument Verification Plate (see page 45).
   a. If the run passes, recalibrations are not necessary.
   b. If the run fails, perform steps 8 through 12 to recalibrate the instrument.



8. Perform an ROI calibration (see page 13).
9. Perform a background calibration (see page 23).
10. Perform an optical calibration (see page 25).
11. Perform a dye calibration (see page 32).
12. Perform an instrument verification run (see page 45).

Notes

Set Up the 7500/7500 Fast System

Set Up the Computer

Refer to the *Applied Biosystems Real-Time System Computer Setup Guide* for information on setting up a computer for use with the 7500/7500 Fast instrument.

Set Up the 7500/7500 Fast Instrument

IMPORTANT! Do not connect the USB cable to the 7500/7500 Fast instrument until you are instructed to do so by this guide.

Materials Required

- Phillips screwdriver (small and thin)
- Power cord

Set Up the 7500/7500 Fast System

1. Prepare the installation site as described in the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide*.
2. Open the access door to the 7500/7500 Fast instrument.
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
   b. Open the access door.
3. Verify that the heated cover assembly is pulled fully toward the front of the instrument. If the 7500/7500 Fast system has a heated cover latch installed, check that the latch is in a locked position.
4. Inspect the instrument for damage caused by the transportation of the 7500/7500 Fast system.

   If the instrument is damaged, record the location and appearance of the damage, then contact Applied Biosystems technical support or your service representative for assistance.
5. Close the access door.
6. Connect the power cord to the 7500/7500 Fast instrument, then to the wall receptacle.

   Note: Power cords for different voltages are provided in the packing kit. Connect the cord with the receptacle appropriate for your voltage, then discard the remaining power cords.
7. Press the power button at the lower right front panel, then wait for the 7500/7500 Fast instrument to start up (about 30 sec).

Notes

8. When the Power status light on the lower left front panel is lit, push the tray door to open it.
9. Remove the packaging plate from the tray and set it aside.
10. Close the tray door, then press the power button again to power off the instrument.

Note: Install any additional hardware.

IMPORTANT! Do not connect the USB cable to the 7500/7500 Fast instrument at this time.

11. Verify that the 7500 software is installed to the computer.

    If the computer does not have the 7500 software, use the Applied Biosystems 7500/7500 Fast Real-Time PCR System Software v2.0 Software CD to install the software.
12. Once you verify that the computer contains the 7500 software, connect the USB cable to the 7500/7500 Fast instrument.

Notes

B

Create a Custom Dye Plate

The Applied Biosystems 7500/7500 Fast Real-Time PCR System can be used to run assays designed with custom dyes (dyes not manufactured by Applied Biosystems). Custom dyes must fluoresce within the 520 to 650 nm spectral range measured by the 7500/7500 Fast instrument.

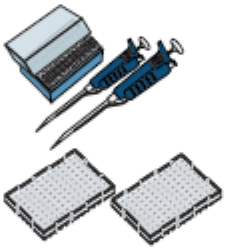
Before Using Custom Dyes

Before using custom dyes with the 7500/7500 Fast instrument, you must:

- Determine optimum dye concentration
- Create a custom dye plate
- Add the custom dye to the software
- Perform a dye calibration (see Chapter 4, "Perform the Dye Calibration.")

Materials Required

Pipettors and pipette tips (200-µL and 1000-µL)



ABI PRISM® Optical 96-Well Reaction Plate and Optical 96-Well Fast Plate

Water, deionized

Tubes (2-mL and 10-mL)

Optical Adhesive Cover or Optical Flat Caps

Safety glasses

Powder-free gloves

Custom dye(s)

Centrifuge with plate adapter

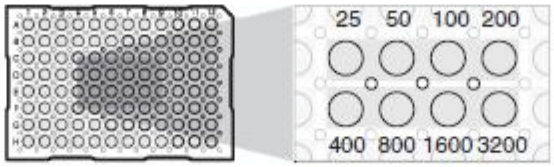
Notes

Appendix B Create a Custom Dye Plate

Determine Optimum Dye Concentration

IMPORTANT! Wear powder-free gloves while creating the dye plate.

1. In the center wells of a 96-well plate, prepare a dilution series of the custom dye (for example, 25, 50, 100, 200, 400, 800, 1600, and 3200 nM) using 50-µL volumes for the 7500 system or 20-µL volumes for the 7500 Fast system.

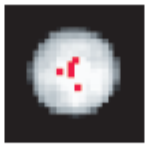


2. Seal the wells of the reaction plate using an optical adhesive cover.
3. Load the prepared reaction plate:
   a. Push the tray door to open it.
   b. Load the dye plate into the plate holder.
   c. Push the tray door to close it.

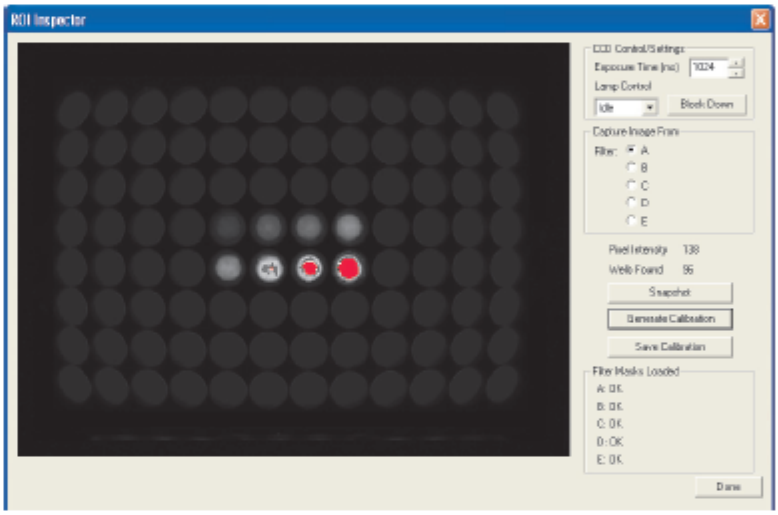
4. In the 7500 software, select **Instrument ▸ Instrument Maintenance Manager**.
5. In the ROI tab of the Instrument Maintenance Manager, click **Start Manual Calibration.**
6. In the ROI Inspector, create the ROI image for each filter, beginning with Filter 1:
   a. In the Exposure Time field, enter **1024**.
   b. Click **Block Up**.
   c. Select **Filter 1**.
   d. Click **Snapshot**.

Notes

e. Inspect the image for saturation.

No Saturation	Normal Saturation	Over Saturation
		
Acceptable	Not Acceptable	

f. Record the coordinate of the well that displays the brightest possible signal without saturation. This well contains the optimal concentration of the custom dye for Filter 1.



7. Repeat steps 6c through 6f for the remaining filters.

8. After you determine the optimum concentration for each filter, determine the optimum concentration for the custom dye:

   a. Compare the results from all filters.

   b. Select the concentration that yields the highest possible signal in all filters, but does not saturate.

Notes

B Appendix B Create a Custom Dye Plate

Unload the Plate

CAUTION **PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. In the ROI Inspector, click **Block Down**.
2. Remove the plate from the instrument:
   a. Push the tray door to open it.
   b. Remove the plate.
   c. Push the tray door to move it into the instrument.

Note: If you cannot open the tray, the sample block may be in its raised position, locking the tray position. To lower the block, click **Move Down**, then click **Block Down**.

3. Click **Done**.

Create a Custom Dye Plate

IMPORTANT! Wear powder-free gloves while creating the dye plate.

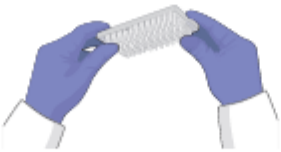
1. Prepare 5 mL (7500 system) or 2 mL (7500 Fast system) of the custom dye at the concentration determined in step 8 on page 77.
2. Pipette 50 µL (7500 system) or 20 µL (7500 Fast system) of the diluted custom dye to all wells of an optical reaction plate.
3. Seal the wells of the reaction plate using an optical adhesive cover.

4. Centrifuge the plate for 2 min at less than 1500 rpm.

IMPORTANT! The custom dye calibration plate must be well mixed and centrifuged.

Notes

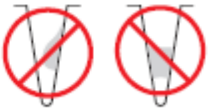
5. Verify that the liquid in each well of the ROI calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.



Correct

Liquid is at bottom of well.

Incorrect



- Not centrifuged with enough force, or
- Not centrifuged for enough time

Add the Custom Dye to the Software

1. In the main screen of the 7500 software, select **Instrument** ▸ **Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager:
   a. In the navigation pane, click **Dye**.
   b. In the Dye screen, select **Custom Dye Calibration**.
   c. Click **Start Calibration**.
3. In the Setup screen of the Dye Calibration dialog box, select a custom dye from the list or create the custom dye as follows:
   a. Click **New Dye**.
   b. In the Dye Manager dialog box, click **New**.
   c. Complete the New Dye dialog box, then click **OK**.

Field/Option	Action
Name	Enter a name for the custom dye.
Wavelength	Enter the wavelength at which the dye fluoresces.
Type	Select: • **Reporter** if the dye works in conjunction with a quencher dye to report an increase of PCR product. • **Quencher** if the dye suppresses the fluorescence of a reporter dye until amplification of PCR product. • **Both** if the dye reports an increase of PCR product without the aid of a quencher dye.

   d. Click **Close**.

Notes

4. In the Setup screen of the Dye Calibration dialog box, enter a temperature setting for the calibration.

   Note: Set the temperature to match the temperature at which you intend to collect data. For example, the temperature for all Applied Biosystems system dyes is 60 °C because data collection for TaqMan® reagents occurs during the 60 °C extension step of the PCR.

5. Select **The custom dye plate is loaded in the instrument**, then click **Next**.

6. In the Run screen, click **Start Run**, then wait for the 7500/7500 Fast instrument to complete the dye calibration.

   Note: If the 7500 software displays messages during the run, troubleshoot the errors as described in "Troubleshoot the Dye Calibration" on page 43.

7. When the 7500/7500 Fast instrument displays the Main Menu, unload the custom calibration plate.

8. Analyze the custom spectral calibration as explained in "Analyze the Calibration Data" on page 39.

Notes

C

Create a Background Plate

Whenever possible, use a background plate that is included with the spectral calibration kit. The plates supplied in the kit contain a buffer that accurately simulates the reagents used for PCR, and, therefore, produces high-quality calibration data. However, if a background plate from a spectral calibration kit is not available, you can create one as described below.

Materials Required

Create a Background Plate

IMPORTANT! Wear powder-free gloves while creating the background plate.

1. Remove an Applied Biosystems 96-Well Optical Reaction Plate or 96-Well Fast Reaction Plate from its box and place it on a clean, dry surface.
2. Aliquot 50 µL (7500 system) or 20 µL (7500 Fast system) of deionized water to each well of the reaction plate.
3. Seal the plate using an optical adhesive cover or optical flat caps.

   Use the plate for background calibration in the same way you use a background plate from the spectral calibration kit. See Chapter 3, "Perform the Background Calibration and Optical Calibration,"

C

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Appendix C Create a Background Plate

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Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at **www.appliedbiosystems.com.**

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