9.1 Purpose

Only suitable and properly operating equipment and software/macros are used for casework and database. This protocol is to ensure that the correct parameters are monitored and equipment maintenance and performance checks are performed on critical equipment and software/macros.

9.2 Responsibility

DNA Section Personnel.

9.3 Equipment Inventory

An inventory for equipment and software/macros is maintained for the DNA section (see appendix for the software/macros). In addition, the Division of Scientific Services maintains a general laboratory inventory.

Annual: Once a calendar within +/- 30 days.

Semi-Annual: Two times a calendar year within 6 months +/-1 month.

Quarterly: Once every three months within +/- 2 weeks

Monthly: Once a month within +/- 7 days

Weekly: Once a week within +/- 3 days.

9.3.1 Broken Equipment Notification

If a piece of equipment is not working properly, notification (visible sign) will be placed on the piece of equipment. The issue will be investigated to try to correct the problem. The equipment will remain out of use until it is deemed operational. Broken, out of service, and in need of repair equipment should be documented on its maintenance log. Notification to the Assistant Director or Deputy Director will be made. Performance checks or QC measures will be completed prior to release for casework, if equipment is critical and can influence the test results.

9.4 Centrifuges

Centrifuges (used for Microcon purification and concentration, see DNA SOPs 2 and 20) will be annually performance checked on site by a qualified vendor. Records will be kept by the Quality Section. If a centrifuge needs repair, it will be put out of use and sent out for repair. Quarterly cleaning of centrifuges will be documented on DNA QR-290.

9.5 Thermometer Calibration Verification

Performance Check each thermometer against a NIST traceable thermometer prior to its first use and annually thereafter.

The actual NIST traceable thermometers used for critical temperature checks and used to performance check other thermometers (i.e. for temperatures such as 56°, 70°, 80º) will also be performance checked annually unless new NIST thermometers are purchased each year to replace the previous year's set. If performance check is to occur in-house, the thermometers will be checked by placing them in boiling water, at room temperature, and in ice or dry ice as necessary by the thermometer's reading capabilities. An acceptable difference between readings from the two thermometers is +/- 2ºC. If readings do not meet acceptable requirements, notification to the DNA TL and a Section Manager will be made and a new thermometer may need to be purchased.

9.5.1 The annual performance check of thermometers (non-NIST) will be done following the directions below:

Each thermometer will be checked against a NIST traceable thermometer prior to its first use and then annually. Calibration may be checked against a certified purchased thermometer by placing the certified thermometer and the thermometer being checked in the freezer, refrigerator, heat block or incubator, leaving them in for at least one hour, and recording the temperature of both thermometers. An acceptable difference between readings from the two thermometers is +/- 2ºC. If the thermometer being calibrated does not read +/- 2ºC of the traceable thermometer, continue to monitor for another day. If required, discard it in the proper manner and replace it with an acceptable one.

Note: If multiple temperatures are set on the equipment depending on the method used (i.e. thermomixers), then the thermometers will be performance checked all those temperatures.

9.5.2 Following the performance checks, mark the top of the thermometers to indicate that they had acceptable results. Please refer to GL-21 and GL-21.3 for documentation. A memo will be written documenting the results of the performance check.

9.6 Refrigerators/Freezers

9.6.1 An assigned calibrated thermometer is inside each refrigerator/freezer.

9.6.2 Log the temperature weekly on the appropriate sheet (DNA QR-200a/b).

9.6.3 If the temperature in the unit is more than ± 5°C (freezer) or ± 3°C (refrigerator) above the set temperature, perform appropriate maintenance measures. Such measures would include readjusting the temperature settings, defrosting the unit, or having the unit serviced.

9.6.4 Record any maintenance or adjustment to each unit on the appropriate log sheet. Please use DNA QR-200a and 200b.

9.7 Pipettes

Pipettes used for DNA amplification will be annually performance checked and calibrated or repaired by an ISO 17025 calibration vendor. Repair and calibrations will be noted on certificate from vendor if performed. Records will be kept by the Quality Section in Qualtrax.

9.7.1 Pipettes are individually identified and tracked (pipette log) either by serial number or numeric designation and are generally assigned to a specific workspace. Pipettes will have colored lab tape to represent their location.

9.7.2 If a pipette in the DNA Unit is found "out of tolerance" during the annual performance check, and is found to be greater than 20% out of tolerance, a QAR will be opened to investigate this incident.

9.8 Constant Temperature Incubators

9.8.1 All ovens have assigned thermometers.

9.8.2 Turn the oven on (if necessary). Set the oven to the desired temperature. After ~20 minutes, check the thermometer.

9.8.3 If the actual temperature of an oven has deviated from the set temperature by more than ±2°C the temperature is readjusted. DNA TL and a Section Manager will be notified.

9.8.4 Each time the oven is used, any maintenance and adjustments are logged on to the appropriate sheet. (See DNA QR-204)

9.9 Mettler Balance

9.9.1 Before each use, verify that the unit is level and clean.

9.9.2 The Mettler must be calibrated once a year by an authorized service representative; Service dates are on the label on the balance. Certificates are kept with the Quality Section.

9.10 Denver Instrument UltraBasic pH Meter

9.10.1 The pH meter is standardized prior to each use. Prior to each new standardization, previous standardizations will be cleared.

9.10.2 Please refer to DNA WI-29 (pH Meter) for further instruction.

9.10.3 Each calibration is logged onto the proper log sheet (please use DNA QR-206a and 206b).

9.10.4 If the instrument does not calibrate properly, follow the troubleshooting guidelines found in the Operational Manual. If problems persist the instrument will be returned to the manufacturer for service. Notify the DNA TL and a Section Manager.

9.10.5 Electrode is stored in a 3M KCL solution. The KCL can be in-house formulated or commercially purchased. Please refer to WI-29 (pH Meter) and QR-267 (3M KCL).

9.11 DNA Thermal Cycler

9.11.1 Log each use of a thermal cycler on the appropriate case amplification worksheet.

9.11.2 Each Thermal Cycler will be performance checked annually by a qualified outside vendor using a NIST traceable temperature measuring device. Certificates will be maintained for each thermal cycler by the Quality Section.

9.11.3 If a Thermal Cycler needs repair or further adjustment, it will be quarantined and sent out for repair. It will be shipped in an appropriate box with padding to prevent any damage. Upon return, the vendor certificate will be maintained to show the thermal cycler left repair acceptable for use and to show the probe used was NIST traceable. An additional performance check will be done before the instrument is reinstated for casework (Please use DNA QR-259).

9.11.4 Each Thermal Cycler's block (including wells) will be cleaned approximately every three months. This is documented on DNA QR-288.

9.12 ABI 3500xL Genetic Analyzer

9.12.1 The ABI 3500xL Genetic Analyzers are serviced annually by factory service contract. The ABI service department is contacted for repairs and the annual service. A performance check will be done following repair or annual service and before the instrument is reinstated for casework. The performance check will include the methodologies that represent the different types of dye sets used for casework. (Document Performance Check by using

DNA QR-260). Acceptable parameters can be found on the DNA Quality Record (QR). If acceptable parameters are not met, notify DNA TL and a Section Manager.

9.12.2 During periods of operation, additional maintenance is performed on the ABI 3500xL Genetic Analyzers and is documented on the appropriate quality record (QR-318 and kept in a maintenance binder by the DNA section. Please refer to DNA SOP-38 for more detailed information on maintenance and cleaning.

9.13 ABI 7500 Real-Time Instrument Maintenance

9.13.1 The ABI 7500 Real-Time instruments are serviced annually by factory service contract. The ABI service department is contacted for repairs and the annual service. Records are maintained in 7500 Performance Check Notebook.

9.13.2 Annually: Instrument performance is verified following the annual service using an ABI TaqMan RNase P verification plate per manufacturer's protocols and documented on DNA QR-262. Records are maintained in Performance Check Notebook.

9.13.3 During periods of operation, additional maintenance is performed on the ABI 7500 instruments by the Division of Scientific Services and is documented on the appropriate log sheet (DNA QR-18).

9.13.4 Approximately every 2 months: background check and block cleaning as necessary (DNA QR-18).

9.13.5 Semi-annually (twice a year): spectral calibrations (Pure Dye) per manufacturer's protocols. (One is performed in association with the annual service). Documented on DNA QR-18.

9.13.6 A performance check will be done before the instrument is reinstated for casework as follows:

Following the annual preventative maintenance (performed by an ABI service engineer), the performance check is as described in 9.13.2 and is documented using DNA QR-262. Following an instrument repair (performed by an ABI service engineer at any other time that year) or following the spectral calibrations (Pure Dye) (performed by laboratory personnel at the 6-month interval following the annual PM), the performance check is done by running a "Quality Controlled Passed" set of DNA standards and a no template control and documented using DNA QR-34 for Quantifiler Trio.

9.13.7 Please refer to ABI 7500 User's Manual for instructions for performing maintenance procedures.

9.13.8 Acceptable parameters can be found on the DNA Quality Record(s) (QR). If acceptable parameters are not met, notify DNA TL and a Section Manager.

9.14 BSD Duet/BSD Plus

Calibration/Preventative Maintenance occurs at least once a year. This may be performed by an analyst or vendor. A performance check is completed after the instrument has been serviced or moved and before the instrument is used for casework. See DNA SOP-28 and use DNA QR-265 for performance checking and DNA QR-296 for maintenance documentation. Acceptable parameters can be found on the DNA Quality Record(s). If acceptable parameters are not met, notify DNA TL and a Section Manager.

9.15 EZ1 Advanced XL

The Qiagen EZ1 Advanced XL Extraction Instrument is serviced annually by factory service contract. The service department is contacted for repairs and the annual service. A performance check is completed after the instrument has been serviced and before its use for casework. For documentation of a performance check use DNA QR-277. Records are maintained in the EZ1 Performance Check/Maintenance/Service Notebook.

Acceptable parameters can be found on the DNA Quality Record(s). If acceptable parameters are not met, notify DNA TL and a Section Manager.

9.16 Heat Blocks

9.16.1 Dry heat blocks are generally set to one or two temperatures (if dual temperature unit). Each unit has a calibrated thermometer (NIST traceable) assigned to it.

9.16.2 Turn on the heat block and wait approximately 30 minutes for the unit to reach the set temperature.

9.16.3 If the unit is not at the set temperature, wait an additional 10 minutes to determine if the unit will reach the set temperature.

9.16.4 If the unit is still not at the set temperature, adjust the temperature setting and wait an appropriate amount of time until the temperature becomes stable.

9.16.5 The temperature of the unit is logged each time the unit is used on the appropriate log sheet (QR-201a/b).

9.16.6 Keep a record of any maintenance or adjustment of each unit on the appropriate log sheet (QR-201a/b).

9.17 Hoods

9.17.1 An outside vendor checks laminar flow hoods and other hoods with airflow annually. The UV bulbs in hoods equipped with them are also checked at this time. The laboratory QC manager maintains the documentation of these results.

9.17.2 The pre-filters in the hoods used for DNA extraction, amplification, and PCIA extraction are changed as needed. The pre-filter in the bone/teeth extraction hood is changed after each use and these changes are documented in the appropriate log sheet located on the hood (DNA QR-331). The pre-filter in the bone/teeth PCR Workstation is changed quarterly and these changes are documented in the appropriate log sheet located on the hood (DNA QR-333). HEPA filters are changed as needed in hoods equipped with them.

9.18 Spectrolinkers

9.18.1 The UV bulb intensities in the Spectrolinker XL-1000s and XL-1500 are checked quarterly by performing the self-diagnostic intensity check as described in the operator's manual. Bulbs will be replaced if the intensity falls below 1500 $\mu W/cm^2$. The bulbs should also be visually inspected through the instruments' windows to confirm that the bulbs are on. Maintenance is documented in the appropriate log sheet (DNA QR-332). See DNA WI-41 for further instructions on Spectrolinker maintenance.

9.19 Software/Macros: See the Appendix below for details

9.19.1 Analysis Software/Statistic Macros Performance Check

GeneMarker HID, GeneMapper, GeneMapper IDX, STRmix, Sequencing Analysis, Sequencher, DNA-QR-46 Probability of Kinship Worksheet, Y-Mix, YHRD Database, DNA QR-44 Match Probability of Parentage Worksheet, and DNA QR-307 Deconvolution Workbook are software/macros used in the DNA Unit for analysis and statistics. A set of data with the expected results for each of the above software/macros will be kept on the U: Drive and used to conduct the performance check on the above softwares/macros semi-annually (approximately every 6 months). See Appendix below for corresponding Quality Records for the various performance checks. Further guidance and details can be found on these DNA QRs. QRs can be found in Qualtrax or if they are macro workbooks, on the Shared drive in the Controlled Document Folder under DNA. Acceptable parameters can be found on the DNA Quality Record(s). If acceptable parameters are not met, notify DNA TL and a Section Manager.

9.19.1.1 STRmix Performance Check

For STRmix, performance checks will be run with the same seed using samples from GF. If there is an upgrade or software patch for STRmix, the performance check will follow the recommendation of the manufacturer. The results will be documented on DNA QR-306. Further guidance/direction is located in the DNA QR-306 workbook. Acceptable parameters can be found on the DNA Quality Record(s). If acceptable parameters are not met, notify DNA TL and a Section Manager.

9.19.2 Processing Macros Performance Check

For macros used throughout the case flow and database process, a performance check will be performed semi-annually. A DNA analyst (other than the macro's creator) may conduct the performance check during his/her proficiency testing from the beginning of the DNA extraction to issuing the DNA report (if applicable). The results will be documented on DNA QR-292 (nuclear/database). Further guidance and details can be found on this DNA QR. See Appendix below for corresponding macros that will be performance checked on DNA QR-292. DNA QRs can be found in Qualtrax or if they are macro workbooks; on the Shared drive in the Controlled Document Folder under DNA.

For the STRmix Known Input File Generator (DNA QR-304), a performance check will be done semi-annually by an analyst in the Database Unit. Please refer to DNA QR-282 for details. Acceptable parameters can be found on the DNA Quality Record(s). If acceptable parameters are not met, notify DNA TL and a Section Manager.

9.19.3 Software/Macros Updates

After a software/macro update, upgrade or software patch and prior to casework commencing, a performance check will be conducted. For software, performance checks will be conducted by any DNA analysts. For excel macros, performance checks will be conducted by any DNA analyst other than the macro's creator. The results of the performance check will be documented on the appropriate QR (see appendix) and notes will describe reasons the performance check was performed. Acceptable parameters can be found on the DNA Quality Record(s). If acceptable parameters are not met, notify the DNA TL and a Section Manager.

9.19.4 Out of Service Software/Macros Notification

If a software/macro is not working properly, notification will be sent out to the DNA Unit through email by the Assistant Director, or designee. The issue, such as a bug in the macro code, will be investigated to try to correct the problem. The software/macro will remain out of use until it is deemed operational. Appropriate performance check will be conducted by the DNA analysts. For macros, performance checks will be conducted by the DNA analysts and

the macro's creator. Documentation will be made on the appropriate QR sheet. After the issue has been remedied, the Assistant Director, or designee will notify the DNA Unit that the software/macro is fit for use in casework.

9.19.5 CODIS

There will be a quarterly performance check of the CODIS software following the directions on DNA QR-291 and the results will be documented on DNA QR-291. See DNA QR-291 for further details and guidance.

A semi-annual check of the CODIS search parameters will also be performed. An email will be sent by the CODIS administrator or alternate after the completion of this check. The Quality Manager maintains a copy of this email.

The integrity of the backup files will be verified on a quarterly basis. An email will be sent by the CODIS administrator or alternate after the completion of this check. The Quality Manager maintains a copy of this email. The CODIS administrator or alternate maintains all File Hash Comparison Reports in a designated binder.

Appendix

Analysis and Statistic Software/Macros

Name	Description	QR	Location of QR
GeneMapper	ID/IDP and Yfiler data analysis	DNA QR-294	Qualtrax
GeneMarker	Fusion 6C, GF, and GFE data analysis	DNA QR-294	Qualtrax
GeneMapper IDX	Yfiler Plus data analysis	DNA QR-294	Qualtrax
STRmix	Deconvolution and HPD calculation	DNA QR-306	S: Drive
Sequencing Analysis	Mitochondrial DNA Analysis	DNA QR-294	Qualtrax
Sequencher	Mitochondrial DNA Analysis	DNA QR-294	Qualtrax
DNA QR-44 Parentage Worksheet	Match Probability of Parentage Worksheet	DNA QR-280	Qualtrax
Y-Mix/YHRD	Y-STR Statistics	DNA QR-280	Qualtrax
DNA-QR-46 Probability of Kinship Worksheet	Kinship Probability workbook	DNA QR-278	Qualtrax
DNA-QR-307 Deconvolution Workbook – F6C	Deconvolution workbook - F6C	DNA QR-309	Qualtrax
DNA-QR-318 Deconvolution Workbook - GF	Deconvolution Workbook - GF	DNA QR-309	Qualtrax
DNA-QR-319 Deconvolution Workbook - YFP	Deconvolution Workbook - YFP	DNA QR-309	Qualtrax

Processing Macros

Name	Description	QR	Location of QR
DNA-QR-25,26,27 EZ1 Extraction Worksheets	EZ1 Worksheet - Knowns	DNA QR-292	Qualtrax
DNA-QR-25,26,27 EZ1 Extraction Worksheets	EZ1 Worksheet - Non Differentials	DNA QR-292	Qualtrax
DNA-QR-25,26,27 EZ1 Extraction Worksheets	EZ1 Worksheet - Differentials	DNA QR-292	Qualtrax

DNA-QR-314 Male Screen Extraction Worksheet	Extraction - Male Screen	DNA QR-292	Qualtrax
DNA-QR-313 Male Screen Concentration	Concentration Worksheet	DNA QR-292	Qualtrax
DNA-QR-24 Quantifiler Trio Worksheet	Quantifiler Trio Worksheet	DNA QR-292	Qualtrax
DNA-QR-22c,32 Dilution Worksheet and Quant Trio Report	Dilution Worksheet - GF	DNA QR-292	Qualtrax
DNA-QR-323 GlobalFiler Amplification Worksheet	GF Amplification Worksheet	DNA QR-292	Qualtrax
DNA-QR-324 Yfiler Plus Amplification Worksheet	YFP Amplification Worksheet	DNA QR-292	Qualtrax
DNA-QR-325 GlobalFiler Injection/Analysis Worksheet	GF/GFE Injection Worksheet	DNA QR-292	Qualtrax
DNA-QR-328 Yfiler Plus Injection/Analysis Worksheets	YFP Injection Worksheet	DNA QR-292	Qualtrax
DNA QR-326 GlobalFiler Express Amplification Worksheet	GFE Amplification Worksheet	DNA QR-292	Qualtrax
DNA-QR-327 GlobalFiler Injection/Analysis Worksheet - Database	GF Injection Worksheet for DB samples	DNA QR-292	Qualtrax
DNA-QR-20 Staff Search Worksheet	Staff Search	DNA QR-292	Qualtrax
Cross-Comparison & Staff Search Tool	Cross-Comparison/Staff Search	DNA QR-292	Qualtrax
DNA-QR-37 Concordance Checker	Positive Control Concordance Checker	DNA QR-292	Qualtrax
DNA-QR-301 Project Comparison Tool	Project Comparison Tool	DNA QR-292	Qualtrax
DNA-QR-302 Contributor Estimation Worksheet	Contributor Estimation Worksheet	DNA QR-292	Qualtrax
DNA-QR-303 STRmix Secondary Diagnostic Output Review	STRmix Secondary Diagnostics	DNA QR-292	Qualtrax
DNA-QR-304 STRmix Known Input File Generator	CO to STRmix Converter/Input file generator	DNA QR-282	Qualtrax