Initials: RCJ **Date:** 5/7/99

Department of Forensic Biology

Quality Manual

Version 1.0

Initials: RG **Date:** 5/7/99

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I. Introduction

Effective this date, this Quality Manual version 1.0 supersedes all previous Quality Assurance (QA) and/or Quality Control (QC) Manuals in the Department of Forensic Biology at the Office of Chief Medical Examiner (OCME) in New York City. The organization of this manual is according to the DNA Advisory Board (DAB) Guidelines. Where appropriate, references have been made to the Department of Forensic Biology Administrative Manual, Case Management Manual, Forensic Biochemistry Methods Manual, and Protocols for Forensic STR Analysis Manual.

The Quality Manual consists of various sections that address the current (FBI, 1998) DAB standards. The Quality Manual Appendices contain reagent sheets (Appendix A), QC procedures (Appendix B), and a list of usage and maintenance logs (Appendix C) that are currently being used in the laboratory.

A. Reagent sheets

The Department of Forensic Biology documents the preparation of all reagents that are prepared in the laboratory. This documentation is in the form of a reagent sheet that lists the chemical makeup and procedures necessary for the preparation of a given reagent. All current reagent sheets are filed in a series of **Reagent Sheet Binders**. A copy of each reagent sheet has also been included in this manual as Appendix A.

B. Quality Control Procedures

The purpose of a QA program is to insure that the laboratory meets a specified standard of quality. The QA program does this through monitoring, verifying, and documenting the performance of the laboratory. To accomplish these tasks, the Forensic Biology QA program has established a series of QC procedures that are designed to monitor critical aspects of forensic sample analysis in order to insure that the resulting product conforms to the current standards set forth by the DAB and the Scientific Working Group for DNA Analysis Methods (SWGDAM). These QC procedures are listed in Appendix B and are identified by specific QC numbers.

C. Usage and Maintenance Logs

Usage and Maintenance Logs are used by the laboratory to provide documentation of equipment use, calibration and maintenance. This documentation also aids the QA program in identifying trends in equipment operation and analyst performance. This information can assist the QA program in the identification of potential or existing problems of quality. A list of the Usage and Maintenance Logs that are used in the laboratory for this purpose are located in Appendix C. These forms can be accessed on the Forensic Biology computer network.

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II. Goals and objectives

The goals and objectives of the Department of Forensic Biology are listed in the Department of Forensic Biology Administrative Manual (section II.A, Goals and Mission).

III. Organization and management

The organization and management structure of the laboratory are diagramed and described in the Administrative Manual (see section II.D, OCME and Department of Forensic Biology Organizational Structure and Figure 1 within).

IV. Personnel Qualifications and Training

Job descriptions for all laboratory personnel are described in the Administrative Manual (section II.D, OCME and Department of Forensic Biology Organizational Structure). In addition, the Civil Service specifications for each job title are kept in a central filing cabinet located in the laboratory along with personnel transcripts, resumes, and documentation of continuing education and training.

V. Facilities

A. Security

Laboratory and building security are discussed in the Administrative Manual (section III.E.3, Security).

B. Contamination

1. Prevention

Several measures have been taken to prevent contamination problems. The laboratory is divided into physically isolated areas for evidence examination, DNA extraction, pre-amplification (amplification setup) and post-amplification (amplification and DNA typing). Each of these areas has its own dedicated equipment. Samples, once they are accepted into the laboratory, move through these areas in one direction only. Samples are first processed in the evidence examination area. They are then moved to the DNA extraction area. Following DNA extraction, aliquots of each sample are quantitated in the DNA quantitation area. Following DNA quantitation, aliquots of each sample are moved into the pre-amplification area. Here fresh kit reagents are stored and samples are prepared for amplification. Finally, the samples are amplified and typed in the post-amplification area. This laboratory setup helps eliminate cross contamination from amplified DNA

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areas back into non-amplified DNA areas.

To avoid cross contamination between specimens, exemplar samples are processed separately from evidence samples. Also, only one sample is processed at a time using single use disposable supplies whenever possible (eg. pipet tips), and scissors/tweezers are thoroughly cleaned between each sample (see the Protocols for Forensic STR Analysis and Case Management Manuals for additional procedures to avoid cross contamination).

By far the best defense against contamination is the training program for the analysts. The analysts must understand what is happening to the DNA at every step of the procedure. They must understand the rationale behind the laboratory set up and the methods of sample handling, so they are able to prevent problems before they arise. In this way, they are equipped to assess and to modify their individual habits as they practise each test of the training program.

2. Contamination Protocol

Contamination is identified as the presence of a positive signal in the extraction negative sample in the Quantiblot analysis procedure or extraneous bands or alleles in the amplification negative, extraction negative or positive controls during STR analysis. Contamination problems reflect a system failure or contamination of the samples by an outside source. The source may be equipment, reagents, or the working environment. Contamination can either be a single isolated event such as cross contamination between two samples or it can be persistent, such as contamination of a reagent or equipment. Persistent contamination may be sporadic and not appear in each run. To remedy contamination caused by a single isolated event, the appropriate extraction, quantitation, amplification and/or STR analysis is repeated (also see the STR Results Interpretation section in the Protocols for Forensic STR Analysis Manual, STR Results Interpretation).

If the contamination persists or if several laboratory members are experiencing the same contamination, the QA Manager must be notified. The source of contamination should be identified, if possible, and eliminated. To demonstrate the elimination of the persistent contamination, a clean run (see QC155) should be performed. During a clean run, control samples are processed along with a series of negative controls. Negative controls are run at the extraction, amplification, and typing steps. The results from these samples will indicate the area in which contamination appears. By focusing attention on one area at a time, the source or sources of contamination can be systematically eliminated. In addition, recent casework may be reviewed and selected samples may be repeated later to verify the results. The analysts will be informed of any corrective action adopted to prevent the recurrence of the problem.

3. Troubleshooting

Often the source of a contamination problem can be identified on the basis of experience. For

example, in a Quantiblot run, a persistent appearance of a light signal in the extraction negative control or the Standard negative control indicates contamination of the Chelex or the sterile water used during the extraction procedure or contamination by the analyst during extraction. This contamination may represent a build up of DNA in the reagents over the course of many extractions. The weak signal appears when the concentration of DNA in the extraction negative is greater than the threshold of detectability for the hybridization. Generally, fresh reagents will eliminate this problem.

Electrophoresis runs which appear to have the same mixture of DNA types across all the samples, indicate a more serious contamination problem at the level of the instrument or amplification step. If tubes or reagents are contaminated during the pre-amplification set up, the contaminant DNA will be amplified along with the sample. The sample signals may even be overwhelmed by the contaminant. To solve this problem, the pre-amplification room must be cleaned out and the bench washed with a 10% bleach solution. All of the kit reagents must be changed and new reaction tubes must be aliquoted.

In some cases, the source of contamination may be more elusive. Problems which persist may be addressed by performing a clean run (QC155).

4. QC Procedures

In addition to proper technique on the part of the analyst, care must also be taken in the preparation of all in-house reagents and in keeping all apparatus that come in contact with forensic samples free of contamination. To this end, various QC procedures have been developed and are part of routine laboratory operation.

a. reagent preparation

Good cleaning of laboratory glassware is an essential first step in reagent preparation (see QC175). Furthermore, all aliquots of deionized water and TE^{-4} (Tris-EDTA) buffer are first sterilized using an autoclave (see QC115) prior to distribution throughout the laboratory. This procedure protects these reagents from possible bacterial contamination that could later result in the degradation of sample DNA. In addition, autoclaving conditions help to keep these solutions DNA-free since DNA is degraded when subjected to these conditions. Other working reagents that are kept in the laboratory for long periods of time (eg. 0.5 M EDTA) may also be autoclaved to increase their shelf life.

b. equipment decontamination

Various QC procedures have also been developed to help maintain a DNA-free environment at the points of sample contact with the various apparatus used in DNA analysis. A dilute bleach solution (10% Sodium Hypochlorite) is extremely effective in degrading DNA and thus is used for

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general cleanup procedures of equipment and of the laboratory environment (eg. laboratory desks and benches). Regular decontamination procedures with 10% bleach are used for the disinfection of the P30 ELISA Plate Washer (QC235), micropipetman (disinfection before and after calibration; see QC215), microcentrifuges (QC140), thermocyclers (QC290), and biosafety/fume hoods (QC125). Generally, this equipment cleaning is done monthly (see specific QC procedures for more information); documentation of these various decontamination procedures is kept in the Plate Washer Maintenance Log Binder, Micropipette Calibration Log Binder, Centrifuge Maintenance Log Binder, Thermocycler Calibration and Maintenance Log Binder and Biosafety/Fume Hood Maintenance Log Binder, respectively.

VI. Evidence Control

Evidence control, handling and documentation procedures are discussed in section III.E (Evidence Handling Protocols) of the Administrative Manual. These procedures have been designed to ensure the integrity of all physical evidence that enters the laboratory.

VII. Validation

Validation procedures are according to the DAB guidelines that are listed in section III.I (Method Validation Records) of the Administrative Manual.

VIII. Analytical Procedures

A. Introduction

Analytical procedures that are used by the Forensic Biology Laboratory are described in the Biochemistry Methods Manual and Protocols for Forensic STR Analysis Manual. These manuals also include general guidelines for the interpretation of data. References to scientific literature on which these procedures are based are also included in these manuals.

B. Reagents

Reagents that are used for the various analytical procedures in the laboratory are purchased from commercial vendors or prepared in the laboratory. Reagents that are purchased from commercial vendors (eg. calibrator standards for quantitation of human DNA, 30% hydrogen peroxide, sodium dodecyl sulfate, sodium hydroxide, etc.) are used either directly in a given analytical procedure (eg. calibrator standards for quantitation of human DNA, 30% hydrogen peroxide) or in the preparation of in-house reagents (eg. sodium dodecyl sulfate, sodium hydroxide).

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Every reagent that is prepared by the Forensic Biology Laboratory is labeled with the identity of the reagent, date of preparation, and individual preparing the reagent. Also, each reagent has a corresponding **reagent sheet** which includes the identity of the reagent, date of preparation, identity of individual preparing the reagent, reagent lot number, standard batch size, ingredients of the reagent, procedure to follow when preparing the reagent, data log section, and the quality control procedures to be performed before the reagent is released for use into the laboratory (see Appendices A and B). Working copies of the reagent sheets are kept in the **Quality Control Reagent Binders**.

1. Lot Numbers

Most reagents are assigned a lot number beginning with "1". Subsequent lots increase in numerical order (eg. ... 51, 52, 53... etc.). Several reagents that are usually made fresh for a given procedure are not assigned lot numbers. Nevertheless, the first use of each new lot/shipment of reagent is subjected to a quality control test. Information about each lot of reagent is recorded on the corresponding **Reagent Inventory Log** (F185), in addition to the corresponding reagent sheet. The purpose of a reagent inventory log is to keep track of the reagent status and flow within the laboratory. The reagent inventory log indicates the date each reagent was prepared, the lot number of that reagent, the quantity of reagent prepared, and where it is stored. Inventory sheets are kept in the QC Reagent Binders. The reagent sheet indicates the lot number of that reagent and the lot number of the ingredients that were used for making the reagent. The reagent sheets for each lot are also filed in the QC Reagent Binders along with any supporting quality control documentation.

2. Standard Batch Size

Each reagent sheet indicates the standard batch size which is routinely prepared for each lot. The quantities listed in the ingredients section have been calculated for this standard batch. Occasionally, it may be convenient to prepare a batch larger or smaller than the standard batch size. In such cases, the preparer must note the adjusted amount of each ingredient added for preparation of the reagent. If changes in demand persist over time, the reagent sheet may be modified to reflect the new batch size.

3. Ingredients

An ingredient may be either purchased from an outside vendor or prepared in the laboratory. The ingredients required for the preparation of the reagent and the amounts of each ingredient required for the standard batch size are listed at the top of the reagent sheet. When suitable, final concentrations, and/or a tolerance of measurement are also listed next to the amount of a given ingredient. The tolerances of measurement are calculated to define an acceptable range of variation that will not significantly change the final concentration of a given reagent. Also, certain ranges have been adopted based upon recommendations for optimum performance. Volume

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measurements which are made in the appropriate size graduated cylinders and which appear to the eye to be exact, fall well within the ranges of tolerance listed in the ingredients section.

The amount of ingredients used in the making of any reagent is recorded in the data log (see below) and on a **Chemical Log Sheet** which is kept in the **Chemical Log Binder**. Chemical log sheets provide information on reagent inventory and flow within the laboratory.

4. Procedure

The procedure describes how to prepare the solution step by step and includes important notes regarding the safe handling of hazardous chemicals. The completed sheets must document exactly how the solution was prepared. Any deviation from the printed procedure must be clearly documented on the reagent sheet.

5. Data Log

The **Data Log** is where information is recorded about the ingredients used in the preparation of reagents. This information includes the source of the ingredient, lot number of the ingredient, amount of ingredient used, date of preparation, and the identity of the individual preparing the reagent. Reagents prepared in the laboratory may also be listed as ingredients (eg. 20% SDS which is used in the preparation of Quantiblot Hybridization Solution). In those cases, the source is listed as FB (Forensic Biology) and the laboratory lot number is recorded.

6. Quality Control

The quality control section lists the tests to be performed, if any, before the solution is released for use in the laboratory. These test procedures have been assigned QC numbers and names (eg. QC145 Chelex Extraction).

The type and number of quality procedures required to be done on a given reagent is dictated by the nature of that reagent. For example, the QC procedure, QC250 Quantiblot Hybridization, is listed in the quality control section for Quantiblot Wash Solution (see Quantiblot Wash Solution reagent sheet in Appendix B). To evaluate the performance of this component, it is not necessary to amplify and type test samples. Only the quantiblot hybridization procedure is necessary to establish quality of the Quantiblot Wash Solution. On the other hand, the QC procedure for 5% Chelex (QC145) requires an extraction, human DNA quantitation, amplification, and STR analysis of the appropriate controls. The newly prepared 5% Chelex solution is released into the laboratory when all the tests have been passed.

More than one solution may be tested with a given QC procedure. In this case, the quality test must be sufficient for all of the components. For example, if a single run is to be performed for 5% Chelex and Quantiblot Wash Solution, the quality test must begin with the extraction. QC145

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Chelex Extraction is the appropriate test for the Chelex, and the procedure encompasses the hybridization necessary for the wash solution.

7. Documentation

After a quality test has been performed, the supporting documentation is attached to the original solution sheet and submitted for review. If the reagent performance is satisfactory, it will be released for general use in the laboratory. If the reagent fails to meet the standards set forth in the QC procedure, it may be submitted for further testing or discarded.

After a reagent has passed quality control and been released, the reagent sheet and quality control documentation are filed in the appropriate QC reagent binder. If more than one reagent has been quality controlled in a single test run, the original quality control documents will be filed with one solution sheet and a cross referenced on the reagent sheet of the other.

C. Critical Reagents

By definition, "critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary samples in order to prevent unnecessary loss of sample." (FBI, 1998). Thus, all critical reagents in the Forensic Biology Laboratory have a QC procedure listed on each respective reagent sheet. This QC procedure must be done in order for the reagent to be released for use in routine casework analysis.

D. NIST standards

PCR standard reference material for STR analysis is obtained from the National Institute of Standards and Technology (NIST) and tested annually as a quality check on the equipment and procedures that are used by the lab for STR typing. This information is documented in the **PCR NIST Standards Binder**.

IX. Equipment Calibration and Maintenance

A. Introduction

Good equipment calibration and maintenance is essential for establishing confidence in the results that are generated during routine testing of forensic DNA samples. Equipment calibration and maintenance procedures can be subdivided into equipment used for (i) weights and measures, (ii) analytical methods, and (iii) lab personnel safety.

1. Weights and Measures

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a. Temperature

The Department of Forensic Biology monitors the temperatures of all freezers, refrigerators, heat blocks, incubators, and water baths that are used for storage of evidence and all types of casework samples on a daily basis during the work week. Temperature readings are documented in **Temperature Log Binders**. Acceptable temperature readings for each specific apparatus are noted below.

equipment	set temperature	acceptable temperature range	log sheet
freezers	-20°C	-2 to -25°C	F115
	-80°C	-60 to -85°C	F120
refrigerators	4°C	1 to 13°C	F190
56°C heat block	56°C	56 ± 3°C	F135
65°C heat block	65°C	65 ± 3°C	F140
95°C heat block	95°C	95 ± 3°C	F145
100°C heat block	100°C	97°C to 105°C	F150
37°C incubator	37°C	37 ± 3°C	F157
Quantiblot H_2O bath	50°C	50 ± 1°C	F230

Digital thermometers (Fisherbrand Traceable Printing Thermometer), digital hygrometers/thermometers (Fisherbrand Hygrometer/Thermometer), and thermocouple meters (Omega Model HH21) are used to monitor the temperatures of the various equipment. Each of these measuring instruments or probes (eg. thermocouples with the exception of the Type T-brown[1]) are calibrated yearly to National Institute of Standards and Technology (NIST) traceable standards (see QC275 and QC280 methods in Appendix B.2). The date of calibration is documented on the appropriate log sheet (see F165) and filed in the **Temperature Equipment Maintenance Log Binder**. All new instruments must either have proof of calibration (eg.

[1] Type T-brown thermocouples are used in the measurement of -80°C low temperature freezers. A verification of these thermocouples is done yearly (see QC285) since an exact low temperature for the storage of DNA extracts, tissue samples, etc., is not critical, and NIST traceable thermometers are not made for this low temperature range.

documentation of traceability to NIST standards) or be calibrated in the laboratory with an NIST traceable standard (eg. NIST traceable mercury thermometer) prior to being used in the laboratory.

Any additional maintenance performed on refrigerators and freezers is documented in the **Temperature Equipment Maintenance Log Binder**.

b. Balances

The Mettler PJ600 and AE260 (analytical) balances are used to weigh chemicals in the ranges of 1 to 200 g and < 10 g, respectively, for the preparation of all laboratory reagents. Balances are calibrated regularly to NIST traceable standards (see QC120 in Appendix B.2). Documentation of each calibration is kept in the **General Equipment Maintenance Binder**.

c. pH Meter

The pH meter is used to measure the pH of reagents (where applicable) that are prepared in the laboratory. A two pH point calibration of standard solutions is done each time the pH meter is used (see QC245 in Appendix B.2). In addition, a weekly two point calibration is performed and documented in the **pH Log & Water System Binder**. The pH meter must be calibrated at both points before being used for routine use in the laboratory.

d. Micropipettes

Micropipettes are used routinely in the laboratory to measure and dispense accurate volumes of reagents used for a given protocol. All micropipettes are calibrated twice each year by an outside vendor (see QC215 in Appendix B.2). In addition, if at any time there is reason to suspect that a micropipette may not be performing to its specification, a quick gravimetric check may be done by weighing specific volumes of water on the Mettler AE260 analytical balance (QC215). If the micropipette differs significantly from specification, the QA Manager should be notified and the micropipette under question will be removed from laboratory operation and will be sent for calibration with the next outgoing shipment. When possible, spare calibrated micropipettes will be used as temporary replacements for any micropipettes that have been removed by this manner from regular operation. Micropipette calibration is documented in the **Micropipette Calibration QC Log Binder.**

2. Analytical Methods

Equipment that is used for specific analytical methods in the laboratory is also calibrated on a regular basis according to each specific QC procedure as indicated below. Documentation of each calibration and maintenance procedure for each equipment is done on specific equipment log sheets

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(see Appendix C) that are filed in each specific equipment log book. Each log book is located near the equipment under consideration.

3. Lab Personnel Safety

The laboratory has a chemical fume hood and several biological containment hoods that are inspected annually by an outside vendor (see QC125 in Appendix B.2). Documentation of inspections are kept in the **Chemical Fume Hood & Biological Cabinet Maintenance Log Book**.

X. Proficiency Testing

Proficiency testing is done in the laboratory according to DAB guidelines. These procedures are discussed in the Administrative Manual (see section III.G, Proficiency Testing).

XI. Corrective Action

Corrective action is discussed in the Administrative Manual (section III.O, Non Conformity and Corrective Action).

XII. Reports

Written procedures for writing and issuing reports are presented in the Case Management Manual.

XIII. Review

Case review and related issues are discussed in the Administrative Manual (section III.C, Data Analysis and Reporting).

XIV. Safety

The Department of Forensic Biology has a documented environmental health and safety program as listed in the Administrative Manual (section III.L, Safety). This documentation is kept in the **Safety Binders**. The OCME building safety officer conducts at least three inspections each year of the laboratory. Documentation of these inspections is also kept in the Safety Binders.

When preparing in-house reagents, safety stickers are used according to the National Fire

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Protection Association (NFPA) safety code.

XV. Audits

The Department of Forensic Biology Laboratory conducts audits annually in accordance to the DAB guidelines (see section III.N, Quality Audit in the Administrative Manual). Documentation that is generated from audits is kept in a central filing system in the laboratory.

XVI. Subcontractor of Analytical Testing

At this time the Forensic Biology Laboratory does not subcontract work to other laboratories. If and when this situation arises, any laboratory that has been subcontracted must also comply to all of the DAB guidelines described in this Quality Manual. In addition, an appropriate and documented review process will be established by the Department of Forensic Biology to verify the integrity of the data received from the subcontractor (see III.P, Subcontracting in the Administrative Manual).

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Appendix A

Reagent sheets that are used for the documentation of reagents used for Forensic Biochemistry Methods and STR Analysis are listed below in sections 1 and 2, respectively, and are presented in alphabetical order. All of these reagent sheets are included in this appendix.

1. Forensic Biochemistry Methods: Reagent Sheets

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2. Forensic STR Analysis: Reagent Sheets

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Acid Phosphatase Test Reagent (5/3/99) lot number: _____
standard batch size: 100 ml total

Two methods: 1) Sodium α-Napthyl Phosphate & Fast Blue B Salt or 2) AP Spot Test Reagent

Ingredients	final concentration	amount
1) Sodium Acetate, 0.1 M	0.1 M	100 ml
Sodium Alpha-Naphthyl Phosphate	0.1%	50 mg
Fast Blue B Salt	0.1%	50 mg
OR		
2) Acid Phosphatase Spot Test Reagent	2.6%	2.6 g

Procedure
Add the sodium alpha-naphthyl phosphate and fast blue B salt to two separate 50 mL conical tubes, each containing 50 ml of 0.1 M sodium acetate. Mix well.

Aliquot 5mL of each reagent into 15 ml conical tubes. Wrap fast blue B salt tubes with aluminum foil.

Store at -20°C.

OR

Dissolve spot test reagent in 90 ml deionized water. Dilute to 100 ml. Store at -20°C.

Data Log

	source	lot	amount
Sodium Acetate, 0.1 M	_____	_____	_____
Sodium Alpha-Naphthyl Phosphate	_____	_____	_____
Fast Blue B Salt	_____	_____	_____
Spot Test Reagent	_____	_____	_____

Quality Control Test
QC100

serum dilution	result
N	____
1/2	____
1/4	____
1/8	____
1/16	____
1/32	____
1/64	____
Negative	____

Quality Control (pass or fail) _____

made by: _____ date: _____

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Alkaline Substrate Buffer (5/3/99) lot number: _____
standard batch size: 1 L

Ingredients	final concentration	amount
Diethanolamine	1.0 M	97 ml
Sodium Azide	0.02%	0.2 g
Magnesium Chloride ($MgCl_2 \cdot 6H_2O$)	0.5 mM	0.1 g
Hydrochloric Acid, concentrated 12.1 M	-----	as needed

Procedure

Dissolve the diethanolamine, sodium azide, and magnesium chloride in 800 ml deionized water.

Adjust to pH 9.8 with hydrochloric acid.

Bring to 1 L volume with deionized water.

Store at 2-8°C in brown bottle or wrap clear bottle with aluminum foil.

Data Log	source	lot	amount
Diethanolamine	_____	_____	_____
Sodium Azide	_____	_____	_____
Magnesium Chloride	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

QC225 xref(date)_____

Quality Control (pass or fail) _____

made by: _____ date: _____

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Amylase Gel Buffer (5/3/99)
standard batch size: 1 L

lot number: _____

Ingredients	final concentration	amount
Sodium Phosphate, anhydrous, monobasic (NaH_2PO_4)	0.05 M	6.2 g
Sodium Phosphate, anhydrous, dibasic (Na_2HPO_4)	0.05 M	7.8 g
Sodium Chloride	7 mM	0.4 g

Procedure

Add the ingredients to 1 L of deionized water.

Adjust pH to 6.9.

Store at 2-8°C.

Data Log	source	lot	amount
NaH_2PO_4, anhydrous	_____	_____	_____
Na_2HPO4, anhydrous	_____	_____	_____
Sodium Chloride	_____	_____	_____

Quality Control

QC105

Standard	Diameter	Activity
20 units	_____	
2 units	_____	
0.2 units	_____	
0.002 units	_____	
0.0002 units	_____	
Negative	_____	
Saliva stain, N	_____	_____
Saliva stain, 1/10 dilution	_____	_____

Quality Control (Pass or Fail) _____

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Anode Solution (IEF Focusing) (5/3/99) lot number: _____
standard batch size: 250 ml

Ingredients	final concentration	amount
Glacial Acetic Acid	1%	2.5 ml

Procedure

Add the acetic acid to 247.5 ml deionized water.

Store at room temperature.

Data Log	source	lot	amount
Glacial Acetic Acid	_____	_____	_____

Quality Control

QC190 xref (Hb IEF plate lot#) _____

made by: _____ date: _____

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Casein Stock Solution (5/3/99) lot number: _____
standard batch size: 1 L

Ingredients	final concentration	amount
Hammerstein Casein	1%	10 g
Sodium Hydroxide, concentrated 10 N	-----	as needed
Phosphate Buffered Saline	50%	500 ml
Sodium Azide	0.1%	0.1 g

Procedure

Thoroughly dissolve the Hammerstein casein in 500 ml deionized water. Add NaOH to pH 8.0 to help casein go into solution.

Add the PBS and sodium azide.

Store at -20°C in 40 ml aliquots.

Data Log	source	lot	amount
Hammerstein Casein	_____	_____	_____
Sodium Hydroxide	_____	_____	_____
Phosphate Buffered Saline	_____	_____	_____
Sodium Azide	_____	_____	_____

QC225 xref(date) _____

Quality Control (pass or fail) _____

made by: _____ date: _____

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Cathode Solution (5/3/99)
standard batch size: 250 ml

lot number: _____

Ingredients	final concentration	amount
Ethanolamine	1%	2.5 ml

Procedure

Add the ethanolamine to 247.5 ml deionized water.

Store at room temperature.

Data Log	source	lot	amount
Ethanolamine	_____	_____	_____

Quality Contol

QC190 xref (Hb IEF plate lot#)_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:CATHODE

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Coomassie Blue Stain (5/3/99)
standard batch size: 1 L

lot number: _____

Ingredients	final concentration	amount
Methanol	50%	500 ml
Glacial Acetic Acid	10%	100 ml
Brilliant Blue R	0.1% (w/v)	1.0 g

Procedure

Mix together methanol, glacial acetic acid, and 400 ml deionized water.

Add brilliant blue R to the solution and stir for several minutes.

Filter the solution directly into a storage bottle.

Store at room temperature.

Data Log	source	lot	amount
Methanol	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____
Brilliant Blue R	_____	_____	_____

made by: _____ date: _____

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Destain Solution (5/3/99)
standard batch size: 4 L

lot number: _____

Ingredients	final concentration	amount
Methanol	45.5%	1820 ml
Glacial Acetic Acid	9%	360 ml

Procedure

Mix together methanol, glacial acetic acid, and 1820 ml deionized water.

Transfer to a 4 L storage bottle.

Store at room temperature.

Data Log	source	lot	amount
Methanol	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:DESTAIN

Initials: RCJ **Date:** 5/7/99

Dithiothreitol (DTT), 0.05 M (5/3/99) lot number: _____
standard batch size: 40 ml

Ingredients	final concentration	amount
Dithiothreitol	0.05 M	0.31 ± 0.005 g

Procedure

Dissolve 0.31 g DTT in 40 ml sterile deionized water.

Dispense approximately 1 ml aliquots of DTT solution into microcentrifuge tubes.

Label with a four month expiration date.

Store at -20°C.

Data Log	source	lot	amount
Dithiothreitol	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:DTT5M

Initials: RG **Date:** 5/7/99

Erythrocyte Acid Phosphatase (ACP) Reaction Buffer (5/3/99) lot number: _____
standard batch size: 2 L

Ingredients	final concentration	amount
Citric Acid, Anhydrous	5 mM	1.92 g
Sodium Hydroxide	0.01 M	0.8 g

Procedure

Dissolve citric acid and sodium hydroxide in 2 L deionized water.

Adjust the pH to 5.0, if necessary, by adding additional sodium hydroxide.

Store refrigerated at 2-8°C.

Data Log	source	lot	amount
Citric Acid	_____	_____	_____
Sodium Hydroxide	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:ACPRB

Initials: RCJ **Date:** 5/7/99

Esterase D (ESD) Reaction Buffer (5/3/99) lot number: _____
standard batch size: 2 L

Ingredients	final concentration	amount
Sodium Acetate, Anhydrous	0.05 M	8.21 g
Glacial Acetic Acid	-----	as needed

Procedure

Dissolve the sodium acetate in 2 L of deionized water.

Adjust pH to 6.5 with 1% glacial acetic acid.

Store refrigerated at 2-8°C.

Data Log	source	lot	amount
Sodium Acetate, Anhydrous	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:ESDRB

Initials: RCJ **Date:** 5/7/99

Iodine Solution, 0.01 N (5/3/99) lot number: _____
standard batch size: 500 ml

Ingredients	final concentration	amount
1 N Iodine (Iodine-Iodide Solution)	0.01 N	5 ml

Procedure

Mix 1 N iodine with 495 ml deionized water.

Store at room temperature in a brown bottle or aluminum foiled glass bottle.

Data Log	source	lot	amount
Iodine, 1 N	_____	_____	_____

Quality Control Test
QC105

Quality Control (pass or fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:IODINE

Initials: RCJ **Date:** 5/17/99

Isoelectric Focusing Acid Phosphatase (ACP) Plates (5/3/99) lot number: _____
standard batch size: 42 ml (10 plates)

Ingredients	final concentration	amount
Sucrose	11.9%	5.0 g
3% Acrylamide Premix	4.8%	2.0 g
Riboflavin (1.0 mg/5 ml H_20)	0.7%	300 ul
Ampholyte pH 4-8	4.8%	2.0 ml
OR		
Ampholyte pH 4-6	2.4%	1.0 ml
Ampholyte pH 6-8	2.4%	1.0 ml

Procedure
Dissolve the sucrose and 3% acrylamide premix in 40 ml of deionized water.
Add the riboflavin.
Add the ampholytes.
Cast solution on glass plates and allow to polymerize at room temperature. Place plates under UV light overnight.
Wrap in wet towels and seal in Kapak bag. Store at 2-8°C.

Data Log	source	lot	amount
Sucrose	_____	_____	_____
3% Acrylamide Premix	_____	_____	_____
Ampholyte pH 4-8	_____	_____	_____
or			
Ampholyte pH 4-6	_____	_____	_____
Ampholyte pH 6-8	_____	_____	_____

Quality Control Test
QC180

Bands	**Allowable Separation**	**Actual Separation**
B1 to B2	≥8 mm	_____
B2 to A	≥10 mm	_____
A to Hb	≥1 mm	_____

5ul Bands Visible Y N Optimal Volume _____

Quality Control (Pass or Fail) _____
made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:IEFACP

Isoelectric Focusing Esterase D (ESD) Plates (5/3/99) lot number: _____
standard batch size: 42 ml (10 plates)

Initials: BCJ **Date:** 5/6/15

Ingredients	final concentration	amount
Sucrose	11.9%	5.0 g
3% Acrylamide Premix	4.8%	2.0 g
Riboflavin (1.0 mg/5 ml H_20)	0.7%	300 ul
Ampholyte pH 4.5-5.4	4.8%	2.0 ml
HEPES	0.034 M	0.34 g
MOPS	0.11 M	1.00 g

Procedure

Dissolve the sucrose and 3% acrylamide premix in 40 ml of deionized water.
Add riboflavin.
Add the ampholyte, HEPES, and MOPS.
Cast solution on glass plates and allow to polymerize at room temperature. Place plates under UV light overnight.
Wrap in wet towels and seal in Kapak bag. Store at 2-8°C.

Data Log	source	lot	amount
Sucrose	_____	_____	_____
3% Acrylamide Premix	_____	_____	_____
Ammonium Persulfate	_____	_____	_____
Riboflavin	_____	_____	_____
Ampholyte pH 4.5-5.4	_____	_____	_____
HEPES	_____	_____	_____
MOPS	_____	_____	_____

Quality Control Test

QC185

ESD Type	**Bands**	**Allowable Separation**	**Actual Separation**
1	top-bottom	≥3 mm	_____
2-1	top-middle	≥1 mm	_____
	middle-bottom	≥1 mm	_____
5-1	top-middle	≥3 mm	_____
	middle-bottom	≥3 mm	_____

5ul Bands Visible Y N Optimal Volume _____

Quality Control (Pass or Fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:IEFESD

Initials: RCJ **Date:** 5/7/99

Isoelectric Focusing Hemoglobin (Hb) Plates (5/3/99) lot number: _____
standard batch size: 42 ml (10 plates)

Ingredients	final concentration	amount
Sucrose	11.9%	5.0 g
3% Acrylamide Premix	4.8%	2.0 g
Riboflavin (1.0 mg/5 ml H_20)	0.7%	300 ul
Ampholyte pH 3-10	0.95%	0.40 ml
Ampholyte pH 4-6	2.4%	1.0 ml
Ampholyte pH 6-8	2.4%	1.0 ml

Procedure
Dissolve the sucrose and 3% acrylamide premix in 40 ml of deionized water.
Add the riboflavin.
Add the ampholytes.
Cast solution on glass plates and allow to polymerize at room temperature. Place plates under UV light overnight.
Wrap in wet towels and seal in Kapak bag. Store at 2-8°C.

Data Log	source	lot	amount
Sucrose	_____	_____	_____
3% Acrylamide Premix	_____	_____	_____
Riboflavin	_____	_____	_____
Ampholyte pH 3-10	_____	_____	_____
Ampholyte pH 4-6	_____	_____	_____
Ampholyte pH 6-8	_____	_____	_____

Quality Control Test
QC190

Bands	**Allowable Separation**	**Actual Separation**
A to F	>2 mm	_____
F to S	>3 mm	_____
S to C	>6 mm	_____

5ul Bands Visible Y N Optimal Volume _____

Quality Control (Pass or Fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:IEFHB

Initials: RCI **Date:** 5/7/99

Isoelectric Focusing Phosphoglutamase (PGM) Plates (5/3/99) lot number: _____
standard batch size: 42 ml (10 plates)

Ingredients	final concentration	amount
Sucrose	11.9%	5.0 g
3% Acrylamide Premix	4.8%	2.0 g
Riboflavin (1.0 mg/5 ml H_20)	0.7%	300 ul
Ampholyte pH 5-7	4.8%	2.0 ml
EPPS (HEPPS)	0.05 M	0.50 g

Procedure

Dissolve the sucrose and 3% acrylamide premix in 40 ml of deionized water.
Add the riboflavin solution.
Add the ampholyte and EPPS (HEPPS).
Cast solution on glass plates and allow to polymerize at room temperature. Place plates under UV light overnight.
Wrap in wet towels and seal in Kapak bag. Store at 2-8°C.

Data Log	source	lot	amount
Sucrose	_____	_____	_____
3% Acrylamide Premix	_____	_____	_____
Riboflavin	_____	_____	_____
Ampholyte pH 5-7	_____	_____	_____
EPPS (HEPPS)	_____	_____	_____

Quality Control Test

QC195

Bands	**Allowable Separation**	**Actual Separation**
type 2+2-	> 4 mm	_____
type 2-1+	> 6 mm	_____
type 1+1-	> 2 mm	_____

5ul Bands Visible Y N Optimal Volume _____

Quality Control (Pass or Fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:IEFPGM

Initials: RJ **Date:** 5/7/11

Kastle-Meyer (KM) Reagent (5/3/99) lot number: _____
standard batch size: 1 L

Ingredients	final concentration	amount
Phenolphthalin	0.2%	2.0 g
Potassium Hydroxide	0.18 M	10.0 g
Absolute Ethanol (100%)	80%	800 ml
Zinc Dust	-----	variable

Procedure
Dissolve the phenolphthalin in 200 ml deionized water in a aluminum foiled flask.
Add the potassium hydroxide.
Stir until clear.
Add the ethanol.
Add enough zinc dust to cover the bottom of bottle.
Store at 2-8°C in a dark bottle.

Data Log	source	lot	amount
Phenolphthalin	_____	_____	_____
Potassium Hydroxide	_____	_____	_____
Ethanol	_____	_____	_____
Zinc Dust	_____	_____	_____

Quality Control Test
QC200

Reagent Sensitivity

whole blood dilution	Before 3% H_2O_2	After 3% H_2O_2
N	_____	_____
1/10	_____	_____
1/100	_____	_____
1/1,000	_____	_____
1/10,000	_____	_____
1/100,000	_____	_____
1/1,000,000	_____	_____
Negative	_____	_____

Quality Control (Pass or Fail) _____

made by : _____ date:_____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:KM

Initials: RCl **Date:** 5/7/99

Leucomalachite Green (LMG) Reagent (5/3/99) lot number: _____
standard batch size: 250 ml

Ingredients	final concentration	amount
Leucomalachite Green (Oxalate Salt)	0.4%	1 g
Glacial Acetic Acid	40%	100 ml
Zinc Dust	-----	5 g

Procedure
Mix together leucomalachite green, glacial acetic acid, 150 ml deionized water, and zinc dust.

Reflux solution by mixing on stir plate until solution is a clear light yellow color. This may take several hours.

Allow to cool and then filter.

Add enough zinc dust to cover the bottom of the bottle.

Store in a dark glass bottle refrigerated at 2-8°C.

CAUTION: HYDROGEN GAS IS GENERATED. DO NOT SEAL BOTTLE TIGHTLY.

Data Log	source	lot	amount
Leucomalachite Green	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____
Zinc Dust	_____	_____	_____

Quality Control Test
QC205
Reagent Sensitivity

whole blood dilution	Before 3% H_2O_2	After 3% H_2O_2
N	_____	_____
1/10	_____	_____
1/100	_____	_____
1/1,000	_____	_____
1/10,000	_____	_____
1/100,000	_____	_____
1/1,000,000	_____	_____
Negative	_____	_____

Quality Control (Pass or Fail) _____

made by : _____ date:_____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:LMG

Initials: **Date:**

Nuclear Fast Red (Red Christmas Tree Stain) (5/3/99) lot number: _____
standard batch size: 1 L

Ingredients	final concentration	amount
Aluminum Sulfate	0.07 M	25.0 g
Nuclear Fast Red	0.05%	500 mg

Procedure

Dissolve the aluminum sulfate in 1 L of warm deionized water and add the nuclear fast red. Stir and allow to cool, then filter.

Store at 2-8°C. The solution is stable for approximately one year.

Data Log	source	lot	amount
Aluminum Sulfate	_____	_____	_____
Nuclear Fast Red	_____	_____	_____

Quality Control

QC150 Pass/Fail _____

made by:_____ date:_____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:NFR

Initials: RCJ **Date:** 5/7/99

P30 ELISA Antisera And Reagents (5/3/99)

Reagents to be tested _____
_____
_____
_____

Data Log	Dilution	source	lot
P30 Antigen		_____	_____
Monoclonal Anti-human P30	_____	_____	_____
Polyclonal Anti-human P30	_____	_____	_____
Alkaline Phosphatase Conjugate	_____	_____	_____
IgG1, Kappa Chain (MOPC 21)		_____	_____
p-Nitrophenol Phosphate Tablets		_____	_____
Alkaline Substrate Buffer		_____	_____
PBS-BSA Solution		_____	_____
Phosphate Buffered Saline		_____	_____
Casein Stock Solution		_____	_____

Quality Control Test
QC225

Quality Control (pass or fail) _____

made by: _____ date: _____

G: USERS: FBIOLOGY:CURRENT:QC:A-RGTSHT:BIOCHEM:P30

Initials: RCJ **Date:** 5/7/99

PBS Solution for P30 ELISA (5/3/99)
standard batch size: 1 L

Ingredients	amount
Phosphate Buffered Saline (PBS) Tablets	5

Procedure

Dissolve the tablets in 1 L of deionized water.

Store at 2-8°C.

Data Log	source	lot	amount
PBS Tablets	_____	_____	_____

QC225 xref(date) _____

made by: _____ date: _____

G: USERS: FBIOLOGY:CURRENT:QC:A-RGTSHT:BIOCHEM:PBS

Initials: RCJ **Date:** 5/7/99

PBS-BSA Solution (5/3/99)
standard batch size: 100 mL

Ingredients	final concentration	amount
Phosphate Buffered Saline (PBS)	n/a	100 ml
Bovine Serum Albumin (BSA)	0.01%	0.01 g

Procedure

Dissolve the BSA in PBS.

Use immediately to prepare stock solution of P30 antigen or store at 2-8°C.

Data Log	source	lot	amount
PBS	_____	_____	_____
BSA	_____	_____	_____

QC225 xref(date) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:PBSBSA

Initials: RCS Date: 5/7/99

Phosphoglutamase (PGM) Reaction Buffer (5/3/99) lot number: _____
standard batch size: 2 L

Ingredients	final concentration	amount
Tris Base	0.1 M	24 g
Magnesium Chloride, Hexahydrate	0.02 M	8 g

Procedure

Mix tris base and magnesium chloride in 2 L deionized water.

Adjust the pH to 8.0, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

Store at 2-8°C.

Data Log	source	lot	amount
Tris Base	_____	_____	_____
Magnesium Chloride	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:PGMRB

Initials: RCJ **Date:** 5/7/09

Phosphoglutamase (PGM) Reaction Mixture (5/3/99) lot number: _____
standard batch size: variable

Ingredients	amount
Glucose 1-Phosphate (with 1% Glucose 1,6-Diphosphate)	3.5 g
NADP Sodium Salt	0.2 g
MTT*	0.3 g

* MTT is [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide]

Procedure

Grind together glucose 1-phosphate (with 1% glucose 1,6-diphosphate), NADP sodium salt, and MTT forming a homogeneous powder. The closed end of a test tube can be used to grind the powder in a beaker.

Equally divide the mixture into approximately 70-75 portions and place aliquots in plastic microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Glucose 1-Phosphate (with 1% Glucose 1,6-Diphosphate)	_____	_____	_____
NADP Sodium Salt	_____	_____	_____
MTT	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:PGMRM

Initials: RCJ **Date:** 5/7/99

Picric Indigo Carmine (PIC) (5/3/99)
(Green Christmas Tree Stain)

lot number: _____

standard batch size: 1 L

Ingredients	final concentration	amount
Picric Acid	0.06 M	13 g
Indigo Carmine	0.34%	3.4 g

Procedure

Dissolve the picric acid in 1 L of warm deionized water; add the indigo carmine and stir overnight.

Store at 2-8°C. The solution is stable for approximately one year.

CAUTION: PICRIC ACID IS EXPLOSIVE WHEN DRY AND SHOULD BE MAINTAINED WITH NOT <10% dH_2O. WEIGH OUT PICRIC ACID WITH NEGLIGIBLE AMOUNT OF WATER IN WEIGH BOAT.

Data Log	source	lot	amount
Picric Acid, Saturated	_____	_____	_____
Indigo Carmine	_____	_____	_____

Quality Control

QC150 Pass/Fail _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:PIC

Initials: RCJ **Date:** 5/7/99

Potassium Cyanide Solution (KCN), 0.05% (5/3/99) lot number: _____
standard batch size: 200 ml

Ingredients	final concentration	amount
Potassium Cyanide	0.05%	0.1 g

Procedure

Dissolve the potassium cyanide in 200 ml of deionized water.

Store at room temperature.

Data Log	source	lot	amount
Potassium Cyanide	_____	_____	_____

QC Test

QC190 xref (Hb IEF plate lot#) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:KCN

Initials: RCJ **Date:** 5/7/99

Saline (0.85% NaCl) (5/3/99) lot number: _____

standard batch size: 10 L

Ingredients	final concentration	amount
Sodium Chloride	0.85%	85.0 g

Procedure

Dissolve the sodium chloride in 10 L of deionized water in a carboy.

Store at room temperature.

Data Log	source	lot	amount
Sodium Chloride	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:SALINE

Initials: RCJ **Date:** 5/7/99

Sodium Acetate, 0.1M (5/3/99) lot number: _____

standard batch size: 1 L

Ingredients	final concentration	amount
Sodium Acetate, Anhydrous	0.1 M	8.21 g
Glacial Acetic Acid	-----	as needed

Procedure

Dissolve the sodium acetate in 1 L of deionized water.

Adjust pH to 5.5 with glacial acetic acid.

Store at room temperature.

Data Log	source	lot	amount
Sodium Acetate, Anhydrous.	_____	_____	_____
Glacial Acetic Acid	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:ACETATE

Initials: RCJ **Date:** 5/7/99

Species Agarose Gel (5/3/99) lot number: _____
(Ouchterlony & Species Crossover Electrophoresis)

standard batch size: 150 ml (variable number of aliquots)

Ingredients	final concentration	amount
Species Tank Buffer	50%	150ml
Sigma Type I Agarose (or equivalent)	1%	3g

Procedure

Mix species tank buffer with 150 ml deionized water.

Dissolve Sigma type I agarose (or equivalent) in the solution by heating on a stir plate.

Once solution is clear, dispense 7 ml aliquots into 20 x 150 mm test tubes.

Allow gels to solidify, then cover tubes with parafilm and store at 2-8°C.

Data Log	source	lot	amount
Species Tank Buffer	_____	_____	_____
Sigma Type I Agarose	_____	_____	_____

Quality Control QC220 or QC255 (Pass/Fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:SPGEL

Initials: RCJ **Date:** 5/7/99

Species Tank Buffer (5/3/99) lot number: _____

standard batch size: 1 L

Ingredients	final concentration	amount
Sodium Barbiturate, C-1V	0.05 M	8.76 g
Diethyl Barbituric Acid (Barbital)	7 mM	1.28 g
Calcium Lactate	0.07M	0.38 g

Procedure

Dissolve sodium barbiturate, barbital, and calcium lactate in 800 ml deionized water.

Adjust the pH to 8.6, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

Dilute to 1 L with deionzed water.

Store at room temperature.

Data Log	source	lot	amount
Sodium Barbirurate	_____	_____	_____
Diethyl Barbituric Acid (Barbital)	_____	_____	_____
Calcium Lactate	_____	_____	_____

Quality Control QC220 or QC255 (pass or fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:TANK

Initials: RCJ **Date:** 5/7/99

Takayama Reagent (5/3/99) lot number: _____
standard batch size: 100 ml

Ingredients	final concentration	amount
Dextrose (Glucose)	0.5%	0.5 g
Sodium Hydroxide	0.25 M	1.0 g
Pyridine	20%	20 ml

Procedure

Dissolve dextrose in 5 ml deionized water.

Dissolve sodium hydroxide in 10 ml deionized water.

Transfer both the dextrose and sodium hydroxide solutions to a flask and add the pyridine.

Dilute solution to 100 ml with deionized water.

Store at 2-8°C in a brown glass bottle.

Data Log	source	lot	amount
Dextrose (Glucose)	_____	_____	_____
Sodium Hydroxide	_____	_____	_____
Pyridine	_____	_____	_____

Quality Control Test
QC265

Results

Positive Control _____
Negative Control _____

Quality Control (pass or fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:TAKA

Initials: RCJ **Date:** 5/7/99

Urea Diffusion Test And Blank Plates (5/3/99) lot number: _____

standard batch size: 613.5 ml (10 plates)

Ingredients	final concentration	amount
Agarose, type 1	1%	6 g
Bromothymol Blue (1.5 g BTB/100 ml dH_20 with two drops of phosphoric acid)	1%	6 ml
urease (300 U/100 ml water)	1.2%	7.5 ml

Procedure

Dissolve the agarose into 600 ml of boiling deionized water.
Add the bromothymol blue solution to the dissolved agarose.
Allow the solution to cool to 50°C.
Separate the solution into two 300 ml portions.
To one portion, add the urease solution.
Dispense 30 ml aliquots of both solutions into 10 cm^2 square petri dishes and allow to solidify.
Store at 2-8°C.

Data Log	source	lot	amount
Agarose, Type 1	_____	_____	_____
Bromothymol Blue	_____	_____	_____
Phosphoric Acid	_____	_____	_____
Urease	_____	_____	_____

Quality Control

QC305

Standard	Diameter	Concentration
urea, 5%	_____	
urea, 0.5%	_____	
urea, 0.05%	_____	
urea, 0.005%	_____	
negative	_____	
urine stain, N	_____	_____
urine stain, 1/10 dilution	_____	_____

Quality Control (Pass or Fail) _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:BIOCHEM:UREA

Initials: RCJ **Date:** 5/7/18

Ammonium Persulfate (0.5g Aliquot) (5/3/99) lot number: _____
standard batch size: ~ 25 tubes x 0.5 g

Ingredients	aliquot	total amount
Ammonium Persulfate (Electrophoresis Grade)	0.5 ± 0.05 g	12.5 ± 1 g

Procedure

NOTE: WHEN WORKING WITH POWDERED AMMONIUM PERSULFATE WEAR GLOVES, EYE PROTECTION, LAB COAT, AND RESPIRATOR FOR SAFETY.

Weigh out 0.5± 0.05 g aliquots of ammonium persulfate and transfer the aliquots to 15 mL conical tubes.

Cap all tubes tightly and label rack containing tubes with contents, lot number, date, and initials.

Store at room temperature.

Data Log	source	lot	amount
Ammonium Persulfate	_____	_____	_____

Quality Control

QC165 STR gel electrophoresis Pass/Fail_____

X ref._____

made by: _____ date: _____

G: USERS: FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR: APS

Initials: RCJ **Date:** 5/21/99

AmpfISTR Blue PCR Reaction Mixture (5/3/99) lot number: _____
standard batch size: ~ 100 tubes x 20 µL

Ingredients	Final Conc.	1 Tube Amount	50 Tubes	100 Tubes
AmpfISTR Blue PCR Reaction Mix	1x	20µL	1000µL	2000µL
AmpliTaq Gold	5U	1µL	50µL	100µL

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN PCR SETUP ROOM. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Add the ingredients to either a 1.5 mL microcentrifuge tube or a 15 mL conical tube using pipetmen dedicated to PCR preparation area only. Vortex and spin the mixture briefly.

While wearing clean gloves, remove sufficient amount of 0.5 mL PCR reaction tubes from the bag and place them in a clean rack designated for the PCR prep room only.

Add 20 µL per tube using a dedicated repeat pipettor or tips with hydrophobic filters.

Cap all tubes and store in a labeled rack away from all sources of DNA.

Store at 2-8°C.

Data Log	source	lot	amount
AmpfISTR Blue reaction mix	_____	_____	_____
AmpliTaq Gold	_____	_____	_____

Quality Control

QC110 Amplification Kits - Only for the first kit of each shipment/lot

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR:BLUE

Initials: RCJ **Date:** 5/7/99

AmpfISTR GREEN PCR Reaction Mixture (5/3/99) lot number: _____
standard batch size: ~ 100 tubes x 20 µL

Ingredients	Final Conc.	1 Tube Amount	50 Tubes	100 Tubes
AmpfISTR Green PCR Reaction Mix	1x	20µL	1000µL	2000µL
AmpliTaq Gold	5U	1µL	50µL	100µL

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN PCR SETUP ROOM. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Add the ingredients to either a 1.5 mL microcentrifuge tube or a 15 mL conical tube using pipetmen dedicated to PCR preparation area only. Vortex and spin the mixture briefly.

While wearing clean gloves, remove sufficient amount of 0.5 mL PCR reaction tubes from the bag and place them in a clean rack designated for the PCR prep room only.

Add 20 µL per tube using a dedicated repeat pipettor or tips with hydrophobic filters.

Cap all tubes and store in a labeled rack away from all sources of DNA.

Store at 2-8°C.

Data Log	source	lot	amount
AmpfISTR Green Reaction Mix	_____	_____	_____
AmpliTaq Gold	_____	_____	_____

Quality Control

QC110 Amplification Kits - Only for the first kit of each shipment/lot

made by:_____ date:_____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR:GREEN

Initials: QCS **Date:** 5/7/99

Bovine Serum Albumin (BSA) Solution, 5 mg/mL (5/3/99) lot number: _____
standard batch size: 25 mL

Ingredients	final concentration	amount
Bovine Serum Albumin	2.5%	125 mg

Procedure

Autoclave a 50 mL glass beaker with a stir bar in it.

Add the BSA to 20 mL of sterile deionized water in the glass beaker.

Stir gently over **very low** heat until the BSA is completely dissolved.

Add the solution to a 50 mL conical tube.

Add sterile deionized water to a final volume of 25 mL.

Aliquot approximately 0.5 mL of BSA solution into 1.5 mL microcentrifuge tubes.

Label each tube with "BSA" and the lot number.

Store at -20°C.

Data Log	source	lot	amount
BSA	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC250 QuantiBlot Quality Control of Solutions (Pass/Fail) _____
QC240 Quad STR/PCR Amplification
QC165 STR gel electrophoresis Pass/Fail_____ X ref._____

made by:_____ date:_____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR:BSA

Initials: Rel **Date:** 5 / 7 / 99

Calibration Control (5/3/99) lot number: _____

Ingredients	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
K562 DNA			7.5 ng/µL	
Yield Gel Loading Buffer	5 X		1 X	----
Sterile Deionized Water	-----		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the K562 DNA received from the manufacturer.

Calculate the final volume according to equation 1.

equation 1 $$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(7.5 ng/µL)}}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

equation 2 (buffer volume) = 0.2(final volume)

Calculate the amount of sterile water to be added according to equation 3.

equation 3 (water volume) = [0.8 * (final volume)] - (initial DNA volume)

Record the buffer and water volumes above.

Initials: RCJ **Date:** 5/7/99

Calibration Control (5/3/99) lot number: _____

To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 200 µL aliquots into sterile 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
K562 DNA	_____	_____	_____
Yield Gel Loading Buffer	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC170 Gel Electrophoresis

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR:CALCON

Initials: RA **Date:** 5/17/99

Cell Lysis Buffer (CLB) (5/3/99) lot number: _____
standard batch size: 2L

Ingredients	final concentration	amount
Sucrose	320mM	219 ± 3g
TRIS base	10mM	2.4 ± 0.1g
Magnesium Chloride, Hexahydrate	5mM	2.0 ± 0.1g
Triton X-100	1.0%	20 ± mL
Hydrochloric Acid, concentrated 12.1 M	-----	variable

Procedure

Dissolve the sucrose, TRIS, and magnesium chloride in approximately 1.5 L deionized water.
Add the Triton to the solution.
Adjust the pH to 7.6 with hydrochloric acid.
Mix well.
Adjust the volume to 2 L with deionized water.
Filter sterilize.
Dispense into sterile 50 mL conical tubes.
Store at 2-8°C.

Data Log	source	lot	amount
Sucrose	_____	_____	_____
TRIS	_____	_____	_____
Magnesium Chloride, Hexahydrate	_____	_____	_____
Triton X-100	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control
QC250 QuantiBlot Quality Control of Solutions- test 20 µL of solution

Pass/Fail _____

final pH: _____ spec: 7.6 ± 0.1

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR:CLB

Initials: RCS **Date:** 5/21/99

Chelex, 5% (5/3/99) lot number: _____
standard batch size: 800 mL

Ingredients	final concentration	amount
Chelex 100	5%	40 g

Procedure

Filter sterilize approximately 900 mL deionized water.

Pour the water into a 1 L bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add 40 g of the Chelex 100 to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the Chelex 100 to a volume of 800 mL using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 mL each into 15 mL conical tubes.

Store at 2-8°C.

Data Log	source	lot	amount
Chelex 100	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control
QC145 Pass/Fail _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR:CHEL5

Initials: RCJ **Date:** 5/2/99

Chelex, 20% (5/3/99) lot number: _____
standard batch size: 500 mL

Ingredients	final concentration	amount
Chelex 100	20%	100 ± 2 g

Procedure

Filter sterilize approximately 600 mL deionized water.

Pour the water into a 500 mL bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add the Chelex to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the Chelex to a volume of 500 mL using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 mL each into 15 mL conical tubes.

Store at 2-8°C.

Data Log	source	lot	amount
Chelex 100	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control
QC160 Pass/Fail _____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR:CHEL20

Initials: RG **Date:** 5/7/99

Chloroform-Isoamyl Alcohol (5/3/99) lot number: _____
standard batch size: 500 mL

Ingredients	final concentration	amount
Chloroform	96%	480 ± 3 mL
Isoamyl Alcohol	4%	20 ± 3 mL

Procedure

NOTE: Use only glass graduated cylinders and containers.

Measure the isoamyl alcohol into a 500 mL brown bottle.

Add the chloroform.

Store at 2-8°C in a flammable materials refrigerator.

Data Log	source	lot	amount
Isoamyl Alcohol	_____	_____	_____
Chloroform	_____	_____	_____

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: CIA

Initials: RCJ **Date:** 5/7/99

Chromogen Solution (5/3/99) lot number: _____
standard batch size: 30 mL

Ingredients	final concentration	amount
Chromogen:TMB	0.2%	60 mg
Absolute Ethanol (100%)	-----	30 mL

Procedure

Bring bottle of chromogen:TMB to room temperature.

Before opening, lightly tap the bottle on the counter to bring its contents to the bottom.

Carefully remove the stopper and reconstitute the chromogen:TMB with the room temperature ethanol.

CAUTION: DO NOT USE ETHANOL STORED IN A METAL CONTAINER; ONLY USE 100% REAGENT GRADE ETHANOL.

Recap the bottle and seal with Parafilm.

Tilt the bottle several times to ensure that all the powder is removed from within the rubber cap.

Shake on an orbital shaker for 30 minutes or longer.

Store at 2-8°C and away from rust.

The solution is stable for six months.

Data Log	source	lot	amount
Chromogen	_____	_____	_____
Ethanol, 100%	_____	_____	_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: CHROM

Initials: RDJ **Date:** 5/2/88

Cofiler PCR Reaction Mixture (5/3/99) lot number: _____
standard batch size: ~ 100 tubes x 20 µL

Ingredients

	Final Conc.	1 Tube Amount	50 Tubes	100 Tubes
Cofiler PCR reaction mix	1x	20µL	1000µL	2000µL
AmpliTaq Gold	5U	1µL	50µL	100µL

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN PCR SETUP ROOM. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.
Add the ingredients to either a 1.5 mL microcentrifuge tube or a 15 mL conical tube using pipetmen dedicated to PCR preparation area only. Vortex and spin the reaction mixture briefly.
While wearing clean gloves, remove sufficient amount of 0.5 mL PCR reaction tubes from the bag and place them in a clean rack designated for the PCR prep room only.
Add 20 µL per tube using a dedicated repeat pipettor or tips with hydrophobic filters.
Cap all tubes and store in a labeled rack away from all sources of DNA.
Store at 2-8°C.

Data Log	source	lot	amount
Cofiler Reaction Mix	_____	_____	_____
AmpliTaq Gold	_____	_____	_____

Quality Control
QC110 Amplification Kits - Only for the first kit of each shipment/lot

made by:_____ date:_____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: COFILER

Initials: RG **Date:** 5/7/99

Deoxynucleotide Triphosphates (2.5 mM) (5/3/99) lot number: _____
standard batch size: ~ 32 tubes x 1000 µL

Ingredients	final concentration	amount
dATP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dCTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dGTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
dTTP, 10 mM, 320 µL/tube	2.5 mM	8000 µL (25 tubes)
Autoclaved, microcentrifuge tubes		~ 32 tubes

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN PCR SETUP ROOM. USE ONLY FILTER PIPET TIPS OR A REPEAT PIPETTOR FOR ALL PIPETTING.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Pool together the manufacturers' shipment of a single dNTP into a 15 ml conical tube. Repeat for all the dNTP's.

Add the pooled dNTP's together in a 50 mL sterile conical tube and mix.

While wearing clean gloves, remove all 1.5 ml eppendorff tubes from the bag and place them in a clean rack designated for the PCR preparation room only.

Aliquot 1000 µL of dNTP mix into each tube.

Once aliquotting is complete, cap all tubes and store in a labeled rack away from all sources of DNA. Store at -20°C.

Data Log	source	lot	amount
dATP	_____	_____	_____
dCTP	_____	_____	_____
dGTP	_____	_____	_____
dTTP	_____	_____	_____

Quality Control

QC240 Quad STR/PCR amplification

QC165 STR gel electrophoresis Pass/Fail _____ X ref. _____

made by:_____ date:_____

G:\USERS\FBIOLOGY\MANUAL\CURRENT\QC\A-RGTSHT.PCR\DNTP

Initials: RG **Date:** 5/7/99

Digest Buffer (5/3/99)

lot number: _____

standard batch size: 2L

Ingredients	Final Concentration	Amount
EDTA, 0.5M	10mM	40 ± 2 mL
TRIS base	10mM	2.4 ± 0.2 g
Sodium Chloride	50mM	5.8 ± 0.4 g
SDS, 20%	2.0%	200 ± 2 mL
Hydrochloric Acid	----	----

Procedure

Add the EDTA, TRIS, sodium chloride, and SDS to approximately 1.5 L deionized water.

Adjust the pH to 7.5 with hydrochloric acid.

Bring up to the final volume with deionized water and mix well.

Measure and record the final pH.

Aliquot into 50 mL centrifuge tubes.

Store at room temperature.

Data Log	source	lot	amount
EDTA, 0.5M	_____	_____	_____
TRIS	_____	_____	_____
Sodium Chloride	_____	_____	_____
SDS, 20%	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control

final pH: _____ specification: 7.5 ± 0.1

QC160 Pass/Fail _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR:

Initials: ecj **Date:** 5/7/21

Dithiothreitol (DTT), 0.39 M (5/3/99) lot number: _____
standard batch size: 7.5 mL

Ingredients	Final Concentration	Amount
Dithiothreitol	0.39 M	0.450 ± 0.005 g
Sterile Deionized Water	__	7.5 mL (Guideline)

Procedure

Add the DTT to approximately 5 mL sterile deionized water in a sterile 15 mL centrifuge tube.

Mix well.

When the DTT is dissolved, bring up to volume with sterile deionized water.

Dispense 500 µL aliquots into sterile 1.5 mL eppendorf tubes. Label with a four month expiration date.

Store at -20°C.

Data Log	source	lot	amount
Dithiothreitol	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC250 QuantiBlot Quality Control of Solutions- Test 20 µL of solution
Pass/Fail_____

made by: _____ date: _____
G: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DTT39M

Initials: RH **Date:** 5/7/99

Dithiothreitol (DTT), 1M (5/3/99) lot number: _____

standard batch size: 20 mL

Ingredients	Final Concentration	Amount
Dithiothreitol	1.0M	3.06 ± 0.05 g
Sterile Deionized Water	-----	-----

Procedure

Add the DTT to approximately 19 mL sterile deionized water in a 50 mL centrifuge tube.

Mix well.

When the DTT is dissolved, bring up to volume with sterile deionized water.

Filter sterilize.

Dispense 250 µL aliquots into sterile 0.5 mL eppendorf tubes. Label with a four month expiration date.

Store at -20°C.

Data Log	source	lot	amount
Dithiothreitol	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC250 Pass/Fail _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DTT1M

Initials: RG **Date:** 7/2/99

EDTA, 0.5M (5/3/99) lot number: _____
(Ethlyenediaminetetraacetic Acid)
standard batch size: 1L

Ingredients	Final Concentration	Amount
EDTA, disodium	0.50 M	186 ± 1 g
Sodium Hydroxide, 10N	------	------

Procedure

Add the EDTA to approximately 500 mL deionized water.

Adjust the pH to 8.0 with sodium hydroxide solution.

Mix well.

When the EDTA is dissolved, adjust the pH to 8.0.

Bring up to volume with deionized water.

Check and record the final pH.

Dispense into 125 mL bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
EDTA	_____	_____	_____
Sodium Hydroxide, 10N	_____	_____	_____

Quality Control

final pH: _____ specification: 8.0 ± 0.1

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: EDTA

Initials: RCJ **Date:** 5/7/99

Formamide, Deionized (5/3/99)
standard batch size: ~36 tubes x 1300 µL

lot number: _____

Ingredients	**Amount**
Formamide (super pure grade)	50 mL

Procedure

NOTE:

THIS PROCEDURE HAS TO BE PERFORMED UNDER THE CHEMICAL FUME HOOD. FORMAMIDE IS HARMFUL BY INHALATION, INGESTION, AND SKIN ABSORPTION. WEAR GLOVES, EYE GLASSES, AND LAB COAT.

Made sure that you are using a super pure grade of formamide. Super pure formamide has been pretreated with a mixed-bed resin.

Check that the pH is greater than 7.0.

Dispense the deionized formamide into 1.5 mL reaction tubes in aliquots of 1300μL and store up to three months at -15 to -20° C.

Label the tube rack with the lot number, the date of manufacture, and the three month expiration date.

Data Log	source	lot	amount
Formamide	_____	_____	_____

Quality Control
QC034 Capillary electrophoresis Pass/Fail _____

made by:_____ date:_____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: FORMA

Initials: RG **Date:** 5/7/99

Formamide and Loading Buffer (5:1) (5/3/99) lot number: _____

standard batch size: 40 x 1200 µL

Ingredients	Amount	Final Ratio
Formamide	1000 ± 20 µL	83%
Sequencing Loading Buffer	200 ± 10 µL	17%

Procedure

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Label 40 1.5mL reaction tubes.

Add Formamide to each tube. Add blue sequencing buffer to each tube.

Close all tubes and mix.

Store at 2-8°C.

Data Log

	source	lot	amount
Formamide	_____	_____	_____
Sequencing Loading Buffer	_____	_____	_____

Quality Control

QC165 STR gel electrophoresis Pass/Fail_____ X ref._____

made by:_____date:_____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: BLUEFOR

Initials: RCS **Date:** 5/17/99

Hydrogen Peroxide, 3% (5/3/99) lot number: _____
standard batch size: ~90 X 0.2 mL

Ingredients	Final Concentration	Amount
Hydrogen Peroxide, 30%	3 %	1.5 mL ± 0.1 mL
Deionized Water	-----	13.5 mL (guideline)

Procedure

Add hydrogen peroxide to a 15 mL disposable tube.

Add deionized water to a final volume of 15 mL.

Aliquot approximately 130 µl of hydrogen peroxide into 1.5 mL brown microcentrifuge tubes.

Label the rack with a two month expiration date.

Store at 4°C in the dark.

Data Log	source	lot	amount
hydrogen peroxide, 30%	_____	_____	_____

made by: _____ date: _____

G:USERS:FBIOLOGY:MANUAL:CURRENT:QC:A-RGTSHT:PCR:3%H2O2

Initials: RCJ **Date:** 5/7/99

Lambda Marker (5/3/99) lot number: _____

Ingredients	initial concentration (ng/µL)	initial volume (µL)	final concentration	final volume (µL)
Lambda Hind III fragments			20 ng/µL	
Yield Gel Loading Buffer	5 X		1X	----
Sterile Deionized Water	----		----	----

Calculations

Record the initial concentration in ng/µL and the initial volume in µL of the lambda Hind III DNA received from the manufacturer.

Calculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(20 ng/µL)}} \qquad \textbf{equation 1}$$

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

(buffer volume) = 0.2 (final volume) **equation 2**

Calculate the amount of sterile water to be added according to equation 3.

(water volume) = [0.8 * (final volume)] - (initial DNA volume) **equation 3**

Record the buffer and water volumes above.

Initials: RS **Date:** 5/7/99

Lambda Marker (5/3/99) lot number: _____

To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

Procedure

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500µL aliquots into sterile 1.5mL eppendorf tubes.

Store at -20°C.

Data Log	source	lot	amount
Lambda Hind III fragments	_____	_____	_____
Yield Gel Loading Buffer	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC170 Gel Electrophoresis

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: LAMBDA

Initials: RG **Date:** 5/7/99

Phosphate Buffered Saline (PBS)
For Chelex Extraction (5/3/99)

lot number: _____

standard batch size: 4L

Ingredients	amount
Phosphate Buffered Saline (PBS) Tablets	20

Procedure

Dissolve the tablets in 4 liters of distilled water.

Data Log	source	lot	amount
PBS Tablets	_____	_____	_____

Measure and record the final pH.
Dispense into 50 mL centrifuge tubes.
Autoclave at 250°F for 20 minutes.
Store at room temperature.

Data Log	source	lot	amount
PBS Tablets	_____	_____	_____

Quality Control

final pH: _____ spec: 7.5 ± 0.1

QC160 Pass/Fail _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: PBSCHE

Initials: RCJ **Date:** 2/1/99

Positive Control - QUAD (5/3/99)
standard batch size ~50 tubes

lot number: _____

Procedure

Collect EDTA blood from a volunteer. Prepare bloodstains from this blood sample.

Extract 3x3 cm portions of the dried bloodstain using the organic extraction procedure in the RFLP manual.

Pool extracts and determine the concentration of DNA using the quantiblot method. Dilutions of up to 1/1000 should be submitted.

Dilute the pooled extract with TE^{-4} to a concentration of 1.25-2.5ng/20ul. Perform QUAD amplification and typing on the pooled extract.

Aliquot pooled extract in microcentrifuge tubes.

Data Log	Source	Quantiblot Results (Pooled fractions)	Dilution	Total Volume	# Aliquots
EDTA Blood	_____	_____	_____	_____	_____

	Source	Lot	Amount
TE^{-4}	_____	_____	_____

Quality Control

QC031- QUAD STR/PCR Amplification

QC pass/fail _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR:PE

Initials: RCJ **Date:** 5/7/99

Primer, DYS19/1 (50 pM/μL) (5/3/99) lot number: _____

Physical data

Sequence 5' CTA CTG AGT TTC TGT TAT AGT 3' NED

Ingredients	amount in pmoles	final concentration	volume dH_2O (μL)
DYS19/1 primer		50 pM/μL	-----
Sterile Deionized Water	-----	-----	

Calculations

Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

Add the sterile water to the original primer tube. Mix well.

Dispense 200 μL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer 19/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1μL of solution Pass/Fail_____

QC240 PCR Amplification (Y STR) and electrophoresis Pass/Fail_____

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DYS19-1

Initials: RG **Date:** 5/4/99

Primer, DYS19/2 (50 pM/μL) (5/3/99) lot number: _____

Physical data

Sequence 5' ATG GCA TGT AGT GAG GAC A 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (μL)
DYS19 / 2 primer		50 pM/μL	-----
Sterile Deionized Water	-----	-----	

Calculations

Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

Add the sterile deionized water to the original primer tube. Mix well.

Dispense 200 μL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer DYS19/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1μL of solution Pass/Fail_____

QC240 PCR Amplification (Y STR) and electrophoresis Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DYS19-2

Initials: RCJ **Date:** 5/7/99

Primer, DYS389/1 (50 pM/μL) (5/3/99) lot number: _____

Physical data

Sequence 5' CCA ACT CTC ATC TGT ATT ATC T 3' NED labelled

Ingredients	amount in pmoles	final concentration	volume dH_2O (μL)
DYS389/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations

Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

Add the sterile water to the original primer tube. Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer DYS389/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail_____

QC240 PCR Amplification (Y STR) and electrophoresis Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DYS389-1

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DYS389-1

Initials: RCJ **Date:** 5/7/99

Primer, DYS389/2 (50 pM/μL) (5/3/99) lot number: _____

Physical data

Sequence 5' TCT TAT CTC CAC CCA CCA GA 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (μL)
DYS389/2 primer		50 pM/μL	-----
Sterile Deionized Water	-----	-----	

Calculations

Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

Add the sterile water to the original primer tube. Mix well.

Dispense 200 μL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer DYS389/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1μL of solution Pass/Fail_____

QC240 PCR Amplification (Y STR) and electrophoresis Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DYS389-2

Initials: RCJ **Date:** 5/7/99

Primer, DYS390/1 (50 pM/μL) (5/3/99) lot number: _____

Physical data

Sequence 5' TAT ATT TTA CAC ATT TTT GGG CC 3' FAM labelled

Ingredients	amount in pmoles	final concentration	volume dH_2O (μL)
DYS390/1 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations

Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

Add the sterile water to the original primer tube. Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer DYS390/1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail_____

QC240 PCR Amplification (Y STR) and electrophoresis Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DYS390-1

Initials: RSD **Date:** 5/7/99

Primer, DYS390/2 (50 pM/μL) (5/3/99) lot number: _____

Physical data

Sequence 5' TGA CAG TAA AAT GAA CAC ATT GC 3'

Ingredients	amount in pmoles	final concentration	volume dH_2O (μL)
DYS390/2 primer		50 pM/µL	-----
Sterile Deionized Water	-----	-----	

Calculations

Calculate the amount of dH_2O to be added according to this equation.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$

Record the water volume above. Have somebody check the calculation.

Procedure

Add the sterile water to the original primer tube. Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer DYS390/2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail_____

QC240 PCR Amplification (Y STR) and electrophoresis Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: DYS390-2

Initials: RCJ **Date:** 5/7/99

Primer F13A1/1 (50 µm) (5/3/99) lot number: _____

Physical data

Sequence JOE - 5' AT GCC ATG CAG ATT AGA AA 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
F13A1/1	5841.8	29.8	5101.2

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume dH_2O (µL)
F13A1 1 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 5101 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RG **Date:** 5/7/99

Primer, F13A1/1 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer, F13A1 1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail _____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref._____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: F13A1-1

Initials: RCJ **Date:** 5/7/99

Primer, F13A1/2 (50 µM) (5/3/99) lot number: _____

Physical data

Sequence 5' GAG GTT GCA CTC CAG CCT TT 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
F13A1/2	6080.0	34.1	5608.6

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume dH_2O (µL)
F13A1 2 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 5609 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ **Date:** 5/7/99

Primer, F13A1/2 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer F13A1 2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail_____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref._____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: F13A1-2

Initials: RCJ **Date:** 5/7/99

Primer, FES/FPS/1 (50 Mm) (5/3/99) lot number: _____

Physical data

Sequence 5' GG GAT TTC CCT ATG GAT TGG 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
FES 1	6173	32.8	5313.5

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume dH_2O (µL)
FES 1 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 5314 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$ **equation 2**

Record the water volume above.
Have somebody check the calculation.

Initials: RG **Date:** 5/7/99

Primer, FES/FPS/1 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer FES/FPS 1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail _____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref. _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: FES-1

Initials: RCJ **Date:** 5/7/99

Primer FES/FPS/2 (50 Mm) (5/3/99) lot number: _____

Physical data

Sequence FAM - 5' GCG AAA GAA TGA GAC TAC AT 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
FES 2	6179	29.7	4806.6

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume dH_2O (µL)
FES 2 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 4807 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ **Date:** 5/7/99

Primer, FES/FPS/2 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer FES/FPS 2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____.

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail_____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref._____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: FES2

Initials: RCJ **Date:** 5/7/99

Primer TH01/1 (50 µM) (5/3/99) lot number: _____

Physical data

Sequence FAM - 5' GT GGG CTG AAA AGC TCC CGA TTA T 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
TH01 1	7386.1	32.3	4373.1

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume dH_2O (µL)
Th01 1 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 4373 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{\text{(amount in pmoles)}}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RG **Date:** 5/7/99

Primer, TH01/1 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer Th01 1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail _____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref._____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: TH01-1

Initials: RCJ **Date:** 5/7/99

Primer TH01/2 (50 µM) (5/3/99) lot number: _____

Physical data

Sequence 5' GTG ATT CCC ATT GGC CTG TTC CTC 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
TH01 2	7257.8	35.1	4836.2

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume dH_2O (µL)
Th01 2 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 4836 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50} \quad \textbf{equation 2}$$

Record the water volume above.

Have somebody check the calculation.

Initials: RCL **Date:** 5/7/99

Primer, TH01/2 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer Th01 2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail_____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref._____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: TH01-2

Initials: RCJ **Date:** 5/7/99

Primer, VWA/1 (50 Mm) (5/3/99) lot number: _____

Physical data

Sequence JOE - 5' CC CTA GTG GAT GAT AAG AAT AAT CAG TAT 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
VWA 1	9272.0	30.1	3246.3

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume H_2O (µL)
VWA 1 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 3246 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50} \quad \textbf{equation 2}$$

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ **Date:** 5/7/19

Primer, VWA/1 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer VWA 1	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1 µL of solution Pass/Fail_____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref. _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL:CURRENT: QC: A-RGTSGT: PCR: VWA1

Initials: RG **Date:** 5/7/99

Primer, VWA/2 (50 µM) (5/3/99) lot number: _____

Physical data

Sequence 5' GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3'

Oligo	M.W.	µg/ O.D.	pmol/ O.D.
VWA 2	9383.0	29.4	3133.3

Ingredients	initial amount (O.D.)	amount in pmoles	final concentration	volume dH_2O (µL)
VWA 2 primer			50 pM/µL	-----
Sterile Deionized Water	-----	-----	-----	

Calculations

Record the initial amount in O.D. received from the manufacturer.

Calculate the total amount in pmoles according to equation 1.

(Amount in pmoles) = (O.D.) x 3133 **equation 1**

Record the amount in pmoles above.

Calculate the amount of dH_2O to be added according to equation 2.

$$(dH_2O \text{ volume}) = \frac{(\text{amount in pmoles})}{50}$$ **equation 2**

Record the water volume above.

Have somebody check the calculation.

Initials: RCJ **Date:** 5/7/99

Primer, VWA/2 (50 µM) (5/3/99) lot number: _____

Procedure

Add the sterile deionized water to the original primer tube.

Mix well.

Dispense 200 µL aliquots into 1.5 mL microcentrifuge tubes.

Store at -20°C.

Data Log	source	lot	amount
Primer VWA 2	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Calculation checked by _____

Quality Control

QC250 Quantiblot- test 1µL of solution Pass/Fail_____

QC240 PCR Amplification (QUAD STR) and Electrophoresis

Pass/Fail_____ X ref. _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: VWA2

Initials: RCJ **Date:** 5/7/99

Profiler Plus PCR Reaction Mixture (5/3/99) lot number: _____

standard batch size: ~ 100 tubes x 20 µL

Ingredients

	Final Conc.	1 Tube Amount	50 Tubes	100 Tubes
Profiler Plus PCR reaction mix	1x	20µL	1000µL	2000µL
AmpliTaq Gold	5U	1µL	50µL	100µL

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN PCR SETUP ROOM. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.
Add the ingredients to either a 1.5 mL microcentrifuge tube or a 15 mL centrifuge tube using pipetmen dedicated to PCR preparation area only. Vortex and spin the reaction mixture briefly.
While wearing clean gloves, remove sufficient amount of 0.5 mL tubes from the bag and place them in a clean rack designated for the PCR prep room only.
Add 20 µL per tube using a dedicated repeat pipettor or tips with hydrophobic filters.
Cap all tubes and store in a labeled rack away from all sources of DNA.
Store at 2-8°C.

Data Log	source	lot	amount
Profiler Plus reaction mix	_____	_____	_____
AmpliTaq Gold	_____	_____	_____

Quality Control

QC110 Amplification Kits- Only for the first kit of each shipment/lot

made by:_____ date:_____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: PROPLUS

Initials: RC **Date:** 5/7/99

QUAD STR/PCR Reaction Mixture (5/3/99) lot number: _____

standard batch size:50-200 tubes

Ingredients:	Final Concentrtion	1 Tube Amount	50 Tubes	100 Tubes	200 Tubes
10X PCR Buffer II	1X	5 µL	250 µL	500 µL	1000 µL
dNTP's (2.5 mM)	200 mM	4 µL	200 µL	400 µL	800 Ml
sterile dH20	--------	5.90 µL	285 µL	590 µL	1180 µL
BSA (5mg/mL)	160ug/ml	1.6 µL	80 µL	160 µL	320 µL
VWA/1 (50pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
VWA/2 (50pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
THO1/1(50pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
THO1/2 (50pM/µL)	0.22 µM	0.22 µL	11 µL	22 µL	44 µL
F13A1/1 (43pM/µL)	0.25 µM	0.29 µL	17 µL	29 µL	58 µL
F13A1/2 (50pM/µL)	0.25 µM	0.25 µL	17 µL	25 µL	50 µL
FES/1/(50pM/µL)	0.20 µM	0.20 µL	10 µL	20 µL	40 µL
FES/2 (50pM/µL)	0.20 µM	0.20 µL	10 µL	20 µL	40 µL
AmpliTaq (5u/µL)	5 U	1 µL	50 µL	100 µL	200 µL
TOTAL		20 µL	1 mL	2 mL	4 mL

Procedure

NOTE: **ALIQUOT ALL TUBES AT ONE TIME AND IN PCR SETUP ROOM. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.**

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper

Add the ingredients to either a 1.5 mL microcentrifuge tube or a 15 mL centrifuge tube using pipetmen dedicated to PCR preparation area only. Vortex and spin the reaction mixture briefly.

While wearing clean gloves, remove sufficient amount of 0.5 mL tubes from the bag and place them in a clean rack designated for the PCR prep room only.

Add 20 µL per tube using a dedicated repeat pipettor or tips with hydrophobic filters.

Cap all tubes and store in a labeled rack away from all sources of DNA.

Store at 2-8°C.

Initials: RDJ **Date:** 5/7/19

QUAD STR/PCR Reaction Mixture (5/3/99) lot number: _____

Data Log	source	lot	amount
10X PCR Buffer II	_____	_____	_____
dNTP's (2.5 mM)	_____	_____	_____
Sterile dH20	_____	_____	_____
BSA (5mg/mL)	_____	_____	_____
VWA/1 (50pM/µL)	_____	_____	_____
VWA/2 (50pM/µL)	_____	_____	_____
THO1/1 (50pM/µL)	_____	_____	_____
THO1/2 (50pM/µL)	_____	_____	_____
F13A1/1 (43pM/µL)	_____	_____	_____
F13A1/2 (50pM/µL)	_____	_____	_____
FES/1/(50pM/µL)	_____	_____	_____
FES/2 (50pM/µL)	_____	_____	_____
AmpliTaq (5u/µL)	_____	_____	_____

made by:_____ date:_____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: QUADRXN

Initials: RG **Date:** 5/7/99

Quantiblot Citrate Buffer (5/3/99) lot number: _____
standard batch size: 8 L

Ingredients	Final Concentration	Amount
Trisodium Citrate	.06M	147.2 ± 0.2 g
Citric Acid	.025M	43.4 ± 2 g (guideline)

Procedure

Dissolve the sodium citrate in approximately 6 L deionized water in a carboy.

Adjust the pH to 5.0 by addition of citric acid (approximately 40 g).

Adjust the final volume to 8 liters with deionized water using two 4 L graduated cylinders.

Mix well.

Measure and record the final pH.

Store at room temperature.

Data Log	source	lot	amount
Trisodium Citrate	_____	_____	_____
Citric Acid	_____	_____	_____

Quality Control

final pH: _____ specification 5.0 ± 0.2

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: QCITR

Initials: RCJ **Date:** 5/7/99

QuantiBlot DNA Standards (5/3/99)
standard batch size: variable

lot number: _____

Ingredients RCJ	final concentration	amount
DNA Standard A	varies	1000 µl
TE^{-4}, 1X	1X	3000 µl

Procedure

1. Pool the contents of four DNA Standard A tubes (use all one lot number). Each tube contains 250 ul of standard.
2. Vortex to mix thoroughly and centrifuge briefly.
3. Label seven sterile1.5 mL microfuge tubes, A - G.
4. Aliquot 500 µL of 1X TE^{-4} into the six tubes labeled B-G.
5. Tube A: Transfer 1000 µL of DNA Standard A into the tube labeled A. This is now DNA Standard A.

   Tube B: Add 500 µL of DNA Standard A to the 500 µL of 1X TE^{-4} in tube B. Vortex to mix thoroughly/centrifuge briefly.

   Tube C: Add 500 µL of DNA Standard B to the 500 µL of 1X TE^{-4} in tube C. Vortex to mix thoroughly/centrifuge briefly.

   Continue the serial dilution through tube 1G.

6. Store at 2° to 8°C. DNA Standards will be stable for at least 3 months.

The seven DNA Standard tubes will have the following concentrations of human DNA:

DNA Standards		
Standard Tube	Conc (ng/µL)	Quantity (ng/5µL)
1A	2	10
1B	1	5
1C	0.5	2.5
1D	0.25	1.25
1E	0.125	0.625
1F	0.0625	0.3125
1G	0.03125	0.15625

Initials: RG **Date:** 5/7/99

QuantiBlot DNA Standards (5/3/99)

Data Log	source	lot	amount
DNA Standard A	_____	_____	_____
TE^{-4}, 1X	_____	_____	_____

Quality Control
QC250 QuantiBlot Hybridization X-ref: _____

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: QSTD

Initials: RG **Date:** 5/7/99

Quantiblot Hybridization Solution (5/3/99) lot number: _____
standard batch size: 6 L

Ingredients	Final Concentration	Amount
SSPE, 20X	5.0 X	1500 ± 10 mL
SDS, 20%	0.50 %	150 ± 1 mL

Procedure

Combine the SSPE and 4350 mL deionized water into a carboy.

Add the SDS.

Warm the solution until all solids are dissolved.

Mix well.

Dispense into 1 L pre-labeled bottles.

Store at room temperature.

Data Log	source	lot	amount
SSPE, 20X	_____	_____	_____
SDS, 20%	_____	_____	_____

Quality Control

QC250 Quantiblot Hybridization

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: QHYB

Initials: RG **Date:** 5/3/99

QuantiBlot Pre-Wetting Solution (5/3/99) lot number: _____
standard batch size: 4 L

Ingredients	Final Concentration	Amount
NaOH, 10 N	0.4 N	160 ± 10 mL
EDTA, 0.5 M	25 mM	200 ± 10 mL

Procedure

Measure 3640 mL deionized water into a 4 L erlenmeyer flask.

Add 160 mL NaOH and 200 mL EDTA.

Mix well.

Dispense into 1 L pre-labeled bottles.

Store at room temperature.

Data Log	source	lot	amount
NaOH, 10 N	_____	_____	_____
EDTA, 0.5 M	_____	_____	_____

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: PREWET

Initials: RCI **Date:** 5/7/99

Quantiblot Spotting Solution (5/3/99) lot number: _____
standard batch size: 300 mL

Ingredients	Final Concentration	Amount
Pre-Wetting Solution	---	74.85 mL ± 1 mL
Bromothymol Blue, 0.04%	0.00008%	150 µL ± 1 µL

Procedure

Measure 74.85 mL Pre-Wetting Solution into a graduated cylinder and pour into a pre-labeled 100 mL bottle.

Repeat for remaining three 100 mL bottles.

Add 150 µL bromothymol blue to each individual bottles.

Cap and mix well by inverting.

Store at room temperature.

Data Log	source	lot	amount
Pre-Wetting Solution	_____	_____	_____
Bromothymol Blue, 0.04%	_____	_____	_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: SPOT

Initials: RCJ **Date:** 5/7/99

Quantiblot Wash Solution (5/3/99) lot number: _____
standard batch size: 20 L

Ingredients	Final Concentration	Amount
SSPE, 20X	2.5 X	2500 ± 50 mL
SDS, 20%	0.10 %	100 ± 5 mL

Procedure

Add 2500 mL SSPE and 17.4 L deionized water into a carboy.

Add in 100 mL 20% SDS.

Mix well.

Aliquot into five 4L brown, pre-labeled bottles.

Store at room temperature.

Data Log	source	lot	amount
SSPE, 20X	_____	_____	_____
SDS, 20%	_____	_____	_____

Quality Control

QC250 Quantiblot hybridization

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: QWASH

Initials: RCJ **Date:** 5/7/99

Sarkosyl, 20% (5/3/99) lot number: _____
standard batch size: 100mL

Ingredients	Final Concentration	Amount
Sarkosyl	20%	20 ± 0.5g

Procedure

Add the sarkosyl to approximately 75 mL deionized water.

Mix until the solution is completely clear.

Bring up to volume with deionized water.

Filter sterilize.

Dispense into sterile 15 mL tubes.

Store at 2-8°C.

Data Log	source	lot	amount
Sarkosyl	_____	_____	_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: SAR20

Initials: RCJ **Date:** 5/7/99

Sequencing Loading Buffer (5/3/99) lot number: _____
standard batch size: 25 mL

Ingredients	Final Concentration	Amount
500mM EDTA, pH8.0	25 mM	1.25 ± 0.05 mL
Blue Dextran	50 mg/mL	1250 mg ± 10 mg

Procedure

NOTE: PREPARE AWAY FROM AMPLIFIED DNA TO MINIMIZE CONTAMINATION. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper.

Pipette EDTA into a 25 mL cylinder. Fill up to 25 mL using deionized water.

Decant into an 100 mL Erlenmeyer flask. Add Blue Dextran. Stir at room temperature until dissolved.

Label 25 1.5 mL reaction tubes.

Add 1000 μL of the sequencing loading buffer to each tube. Close all tubes.

Store at 2-8°C.

Data Log

	source	lot	amount
500 mM EDTA, pH8.0	_____	_____	_____
Blue Dextran	_____	_____	_____

Quality Control

QC165 STR gel electrophoresis Pass/Fail_____ X ref. _____

made by:_____date:_____

G:USERS:FBIOLOGY: MANUAL:CURRENT: QC: A-RGTSHT:PCR: LBSEQ

Initials: RCJ **Date:** 5/7/99

Sodium Acetate, 0.2 M (5/3/99) lot number: _____
standard batch size: 250mL

Ingredients	Final Concentration	Amount
Sodium Acetate, Anhydrous	0.2M	4.1 ± 0.1g

Procedure

Slowly add the sodium acetate to approximately 200mL deionized water.

Mix well.

Bring up to volume with deionized water.

Mix well.

Dispense into 100mL bottles.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

Data Log	source	lot	amount
Sodium Acetate, Anhydrous	_____	_____	_____

Quality Control

QC250 QuantiBlot Quality Control of Solutions- Test 20 µL of solution

Pass/Fail_____

made by: _____ date: _____

G:USERS:FBIOLOGY: MANUAL:CURRENT: QC: A-RGTSHT:PCR: NAACET

Initials: RG **Date:** 5/7/99

SDS, 0.1% (5/3/99) lot number: _____
standard batch size: 20 L

Ingredients	Final Concentration	Amount
SDS, 20%	0.1 %	100 ± 10 mL
OR		
SDS (solid)	0.1%	20 ± 0.2 g

Procedure

Add approximately 15 L of deionized water into a 20 L carboy.

Add 100 mL 20% SDS.

Mix .

Bring up to a final volume of 20 L with deionized water.

Mix.

Store at room temperature.

OR

Warm approximately 750 ml deionized water on a stirring hot plate.

Add the SDS (solid) and allow to dissolve.

When the solution is clear, bring up to a final volume of 20 L with deionized water.

Store at room temperature.

Data Log	source	lot	amount
SDS, 20%	_____	_____	_____
SDS (solid)	_____	_____	_____

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: 1%SDS

Initials: RCJ **Date:** 5/7/99

SDS, 10% (5/3/99) lot number: _____

standard batch size: 100mL

Ingredients	Final Concentration	Amount
Sodium Dodecyl Sulfate	10%	10.0 ± 0.3g

Procedure

CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION.. WEAR GOGGLES FOR EYE PROTECTION.

Dissolve the 50mL of SDS 20% in approximately 50mL deionized water.

Warm the solution until all the solids have dissolved and the solution is clear.

Filter sterilize the warm solution.

Dispense into sterile 100mL bottles.

Store at room temperature.

Data Log	source	lot	amount
Sodium Dodecyl Sulfate, 20%	_____	_____	_____

Quality Control

QC250 QuantiBlot Quality Control of Solutions- Test 20 µL of solution

Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY MANUAL CURRENT: QC: A-RGTSHT: PCR: 10%SDS

Initials: RG **Date:** 5/7/15

SDS, 20% (5/3/99) lot number: _____

standard batch size: 1L

Ingredients	Final Concentration	Amount
Sodium Dodecyl Sulfate	20 %	200 ± 5 g

Procedure

CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION. WEAR GOGGLES FOR EYE PROTECTION.

Warm approximately 750 mL deionized water on a stirring hot plate.

Add a fraction of the SDS, allowing the solids to dissolve before adding more.

Add the SDS until it is all in solution.

When the solution is clear, bring up to volume with deionized water.

Filter sterilize the warm solution.

Store at room temperature.

Data Log	source	lot	amount
Sodium Dodecyl Sulfate	_____	_____	_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC:A-RGTSHT: PCR: 20%SDS

Initials: RCd **Date:** 5/7/99

SSPE, 20X (5/3/99) lot number: _____
standard batch size: 8 L

Ingredients	Final Concentration	Amount
EDTA, Disodium Salt	20. mM	59.6 ± 1.4 g
Sodium Hydroxide, 10N	-----	80 ± 10 mL (guideline)
Sodium Phosphate, Monobasic	200 mM	220 ± 6 g
Sodium Chloride	3.6 M	1680 ± 20 g

Procedure

Dissolve the EDTA in approximately 6 liters deionized water (use SSPE carboy).
Adjust the pH to approximately 6.0 with 10N sodium hydroxide to help dissolve the EDTA.
Add the sodium phosphate first and then the sodium chloride.
Adjust the pH to 7.4 with 10N sodium hydroxide (about 80 mL).
Adjust the final volume to 4 liters with deionized water using two 4L graduated cylinders.
Measure and record the final pH.
Store at room temperature.

Data Log	source	lot	amount
EDTA, Disodium Salt	_____	_____	_____
Sodium Hydroxide, 10N	_____	_____	_____
Sodium Phosphate, Monobasic	_____	_____	_____
Sodium Chloride	_____	_____	_____

Quality Control

final pH: _____ specification 7.4 ± 0.2

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: SSPE

Initials: RCJ **Date:** 5/7/99

Stain Extraction Buffer (5/3/99) lot number: _____
standard batch size: 1 L

Ingredient	Final Concentration	Amount
EDTA, 0.5M	10 mM	20 ± 1 mL
TRIS-HCl, 0.1M - pH 7.8	10 mM	100 ± 0.5 mL
Sodium Chloride	100 mM	5.8 ± 0.2 g
Dithiothreitol	33.9 mM	5.227 ± 0.008 g
SDS, 20%	2.0%	100 ± 3 mL
Sodium Hydroxide, 10N	-----	-----

Procedure

Add all the ingredients except for the SDS to approximately 400 mL deionized water.

Mix well.

Adjust the pH to 8.0 with 10N NaOH. Record the pH.

Add the SDS. Mix well.

Bring up to the final volume with deionized water.

Dispense 10 mL into sterile 15 mL tubes.

Store at 2-8°C.

Data Log	source	lot	amount
EDTA, 0.5M	_____	_____	_____
TRIS-HCl, 0.1M - pH 8.0	_____	_____	_____
Sodium Chloride	_____	_____	_____
Dithiothreitol	_____	_____	_____
SDS, 20%	_____	_____	_____
Sodium Hydroxide, 10N	_____	_____	_____

Quality Control
final pH: _____ specification 8.0 ± 0.2
QC250 QuantiBlot Quality Control of Solutions- Test 20 µL of solution
Pass/Fail_____
made by: _____ date: _____
G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: SEB

Initials: RCJ **Date:** 5/7/99

Sterile Deionized Water (5/3/99) lot number: _____

standard batch size: 2 L

Procedure

Filter sterilize 2 L of deionized water.

Aliquot 10 mL each into 15 mL centrifuge tubes (200 tubes).

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Quality Control

QC250 Quantiblot Quality Control of Solutions- Test 20 µL of Solution
Pass/Fail _____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: STERH2O

Initials: RG **Date:** 5/7/99

TRIS-EDTA (TE^{-4}), 1X (5/3/99) lot number: _____
standard batch size: 500mL

Ingredients	Final Concentration	Amount
TRIS-HCl, pH 8.0, 1 M	10 mM	5.0 ± 0.3 mL
EDTA, 0.5 M	0.1mM	100± 2µL
OR		
TE, 100X	1.0X	5.0 ml

Procedure

Add the TRIS and EDTA to 495 mL deionized water. Mix well and filter.

Dispense into 15 mL sterile centrifuge tubes.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

OR

Add TE, 100X to 495 ml deionized water.

Dispense into 15 ml sterile centrifuge tubes.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
TRIS-HCl, pH 8.0, 1 M	_____	_____	_____
EDTA, 0.5 M	_____	_____	_____
TE, 100X	_____	_____	_____

Quality Control
final pH: _____ specification: 7.4 ± 0.2

QC250 QuantiBlot Quality Control of Solutions- Test 20 µL of solution

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: TE1X

Initials: RG **Date:** 5/17/99

TRIS-HCl, 1M - PH 8.0 (5/3/99) lot number: _____
standard batch size: 500 mL

Ingredients	Final Concentration	Amount
TRIS base	1.00 M	60.5 ± 0.1 g
Hydrochloric Acid	------	------

Procedure

Add the TRIS to approximately 400 mL deionized water.

Mix well.

Adjust the pH to 8.0 with concentrated hydrochloric acid.

Bring up to final volume with deionized water.

Measure and record the final pH.

Prepare a 1:100 dilution (10 mM TRIS-HCl) by mixing 1 mL TRIS-HCl solution and 99 mL deionized water.

Measure and record the pH of the dilution.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
TRIS	_____	_____	_____
Hydrochloric Acid	_____	_____	_____

Quality Control

final pH: _____ spec: 8.0 ± 0.1 1:100 pH: _____ spec: 8.0 ± 0.1

made by: _____ date: _____

G:USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: TRIS

Initials: RG **Date:** 5/7/99

Tris Sodium EDTA (1X TNE) (5/3/99) lot number: _____

standard batch size: 100 mL

Ingredients	Final Concentration	Amount
TNE, 10X	1.0X	10.0 ± 0.3 mL

Procedure

Add the TNE to approximately 80 mL deionized water.

Bring up to the final volume with deionized water.

Dispense into a 125 mL bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log	source	lot	amount
TNE, 10X	_____	_____	_____

Quality Control

QC250 QuantiBlot Quality Control of Solutions- Test 20 µL of solution

Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: TNE1X

Initials: RG **Date:** 5/7/99

Urea (10.8 g·Aliquot-377 Sequencer) (5/3/99) lot number: _____

standard batch size: ~ 25 tubes x 10.8 g

Ingredients	**Aliquot**	**Total Amount**
Urea (Electrophoresis Grade)	10.8 ± 0.1 g	450 ± 4 g

Procedure

NOTE: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, AND LAB COAT FOR SAFETY.

Fill out chemical logbook.

Using small weigh boat, weigh 10.8 ± 0.1 g aliquots of urea.

Transfer the aliquots to labeled 50 mL conical tubes.

Cap all tubes tightly and label rack containing tubes with contents, lot number, date, initials, and safety data.

Store at room temperature.

Data Log	source	lot	amount
Urea	_____	_____	_____

Quality Control

QC165 STR gel electrophoresis Pass/Fail_____

X ref._____

made by:_____date:_____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: UREA10

Initials: RG **Date:** 5/7/99

Urea (18 g Aliquot-377 Sequencer) (5/3/99) lot number: _____

standard batch size: ~ 25 tubes x 18 g

Ingredients	**Aliquot**	**Total Amount**
Urea (Electrophoresis Grade)	18 ± 0.1 g	450 ± 4 g

Procedure

NOTE: WHEN WORKING WITH POWDERED UREA WEAR GLOVES, EYE PROTECTION, AND LAB COAT FOR SAFETY.

Fill out chemical logbook.

Using small weigh boat, weigh 18 ± 0.1 g aliquots of urea.

Transfer the aliquots to 50 mL conical tubes.

Cap all tubes tightly and label rack containing tubes with contents, lot number, date, initials, and safety data.

Store at room temperature.

Data Log	source	lot	amount
Urea	_____	_____	_____

Quality Control

QC165 STR gel electrophoresis Pass/Fail_____

X ref._____

made by:_____date:_____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: UREA18

Initials: RG **Date:** 5/7/99

Y1 STR/PCR Reaction Mixture (5/3/99) lot number: _____

standard batch size:50-200 tubes

Ingredients:	Final Concentration	1 Tube Amount	50 Tubes	100 Tubes	200 Tubes
10X PCR Buffer II	1X	5 µL	250 µL	500 µL	1000 µL
dNTP's (2.5 mM)	200 µM	4 µL	200 µL	400 µL	800 µL
sterile dH20	--------	7.4µL	370 µL	740 µL	1480µL
BSA (5mg/mL)	160µg/mL	1.6 µL	80 µL	160 µL	320 µL
DYS19/1 (50pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS19/2 (50pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS390/1 (50pM/µL)	0.24µM	0.24 µL	12 µL	24 µL	48 µL
DYS390/2 (50pM/µL)	0.24 µM	0.24 µL	12 µL	24 µL	48 µL
DYS389/1 (50pM/µL)	0.12 µM	0.12 µL	6 µL	12 µL	24 µL
DYS389/2 (50pM/µL)	0.12 µM	0.12 µL	6 µL	12 µL	24 µL
AmpliTaq Gold (5u/µL)	4 U	0.8 µL	40 µL	80 µL	160 µL
TOTAL		20 µL	1 mL	2 mL	4 mL

Procedure

NOTE: ALIQUOT ALL TUBES AT ONE TIME AND IN PCR SETUP ROOM. USING CLEAN GLOVES IS ESSENTIAL; CHANGE THEM AS OFTEN AS NEEDED.

Clean the bench top thoroughly using a 10% bleach solution, and cover it with new bench paper

Add the ingredients to either a microcentrifuge tube or a 15 mL centrifuge tube using pipetmen dedicated to PCR preparation area only.

While wearing clean gloves, remove sufficient amount of tubes from the bag and place them in a clean rack designated for the PCR prep room only.

Vortex and spin briefly. Add 20 µL per 0.2mL tube using a dedicated repeat pipettor or tips with hydrophobic filters.

Cap all tubes and store in a labeled rack away from all sources of DNA.
Store at 2-8°C.

Initials: RCJ **Date:** 5/7/99

Y1 STR/PCR Reaction Mixture (5/3/99) lot number: _____

Data Log	source	lot	amount
10X PCR Buffer II	_____	_____	_____
dNTP's (2.5 mM)	_____	_____	_____
sterile dH20	_____	_____	_____
BSA (5mg/mL)	_____	_____	_____
DYS19/1 (50pM/µL)	_____	_____	_____
DYS19/2 (50pM/µL)	_____	_____	_____
DYS390/1 (50pM/µL)	_____	_____	_____
DYS390/2 (50pM/µL)	_____	_____	_____
DYS389/1 (50pM/µL)	_____	_____	_____
DYS389/2 (50pM/µL)	_____	_____	_____
AmpliTaq Gold (5u/µL)	_____	_____	_____

made by:_____ date:_____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: Y1STR

Initials: RCJ **Date:** 5/7/99

Yield Calibrators (5/3/99) lot number: _____

standard batch size: 5 x 400µl each

Ingredients	Final Concentration	Amount
TE^{-4}, 1X	1X	----
Lambda DNA	----	140 10 µg (guideline)
Yield Gel Loading Buffer	1.25X	3.0 0.5 ml
Sterile Water	----	

Calculations

Stock Solution

Final DNA Concentration	Final Volume	Initial DNA Concentration	Volume Lambda DNA	Volume 1X TE^{-4}
50 ng/µl	2800 µl			

Calibrators

Calibrator	Final DNA Concentration	Stock DNA Concentration	Volume Stock DNA	Volume Water	Volume Buffer
A	300ng/10µl	50ng/µl	1200µl	300µl	500µl
B	200ng/10µl	50ng/µl	800µl	700µl	500µl
C	100ng/10µl	50ng/µl	400µl	1100µl	500µl
D	50ng/10µl	50ng/µl	200µl	1300µl	500µl
E	25ng/10µl	50ng/µl	100µl	1400µl	500µl
F	10ng/10µl	50ng/µl	40µl	1460µl	500µl

Procedure

Each lot of yield calibrators is prepared as a batch of five sets. Each batch requires 2800µl of 50ng/µl stock lambda DNA solution.

Record the concentration in ng/µl of the lambda DNA recieved from the manufacturer under initial DNA concentration.

Initials: RCJ **Date:** 5/7/99

Yield Calibrators (5/3/99) lot number: _____

Procedure

Calculate the volume of lambda DNA required for the stock solution according to equation 1.

$$(\text{volume lambda DNA}) = \frac{(\text{final DNA concentration})(\text{final volume})}{(\text{initial DNA concentration})} \qquad \textbf{equation 1}$$

Calculate the volume of 1X TE^{-4} to add to the stock solution according to equation 2.

$$(\text{volume 1X } TE^{-4}) = (\text{final volume}) - (\text{volume lambda DNA}) \qquad \textbf{equation 2}$$

Prepare the stock solution by diluting the lambda DNA in a sterile centrifuge tube with 1X TE^{-4} and mix well.

Label six sterile eppendorf tubes, one for each of the six yield calibrator levels.

Pipet the appropriate amounts of DNA stock solution and sterile water into the labeled tubes. The combined volume of DNA and water is 1500 µL for each level. Mix well.

Divide each level into five 300µL aliquots, and dispense into labeled, sterile eppendorf tubes.

Add 100µL of yield gel loading buffer to each tube. The final volume of each aliquot is 400µL.

Store at -20°C.

Data Log	source	lot	amount
TE^{-4}, 1X	_____	_____	_____
Lambda DNA	_____	_____	_____
Yield Gel Loading Buffer	_____	_____	_____
Sterile Deionized Water	_____	_____	_____

Quality Control

QC165 Gel Electrophoresis Pass/Fail_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: YCAL

Initials: RG **Date:** 5/7/99

Yield Gel Loading Buffer (5/3/99) lot number: _____
standard batch size: 100 mL

Ingredients	Final Concentration	Amount
Ficoll 400	12.5%	12.5 ± 0.1 g
EDTA, 0.5M	50. mM	10.0 ± 0.1 mL
TAE, 10X	5.0 X	50.0 ± 0.5 mL
SDS, 20%	0.20 %	1.00 ± 0.02 mL
Bromophenol Blue	0.25%	0.25 ± 0.01 g
Xylene Cyanol	0.25%	0.25 ± 0.01 g

Procedure

Combine the Ficoll, EDTA, TAE, and SDS.
Mix well. The solution may need to be heated gently to dissolve the Ficoll.
Add the bromophenol blue and xylene cyanol.
Mix well.
When all the solids are dissolved, bring up to volume using deionized water.
Filter sterilize.
Dispense 1.5 mL aliquots into sterile 1.5 mL eppendorf tubes.
Store at -20°C.

Data Log	source	lot	amount
bromophenol blue	_____	_____	_____
xylene cyanol	_____	_____	_____
Ficoll 400	_____	_____	_____
EDTA, 0.5M	_____	_____	_____
TAE, 10X	_____	_____	_____
20% SDS	_____	_____	_____

made by: _____ date: _____

G: USERS: FBIOLOGY: MANUAL: CURRENT: QC: A-RGTSHT: PCR: LBYG

Initials: RCJ **Date:** 5/7/99

Appendix B

QC procedures used in the OCME Forensic Biology Laboratory are contained in this appendix. These procedures are divided into two parts: 1) General and Analytical Methods, and 2) Calibration and Maintenance. The General and Analytical Methods section refers to QC procedures for the testing of reagents that are used in various analytical methods in the laboratory. Also included in this section are general QC procedures that are used to insure an appropriate laboratory environment for the performance of the various analytical methods. The Calibration and Maintenance section includes QC procedures that are done to monitor and insure the optimum performance of various instruments and apparatus used in the laboratory.

1. QC Procedures: General and Analytical Methods

Initials: RCS **Date:** 5/7/99

2. QC Procedures: Calibration and Maintenance

Initials: RC **Date:** 5/7/99

QC100 Acid Phosphatase Spot Test Reagent

Test Materials:

Acid Phosphatase Spot Test Reagent

Samples

Whole human semen
Deionized water

Procedure

Prepare 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 dilutions of whole human semen with saline.

Prepare dried stains of each dilution (including a neat semen stain) on filter paper.

Perform the spot test on each stain and on a negative control (deionized water) stain as specified in the Biochemistry Methods Manual.

Specifications

Positive results should be obtained on each semen dilution stain.

Negative results must be obtained with the negative control stain.

Documentation

Write test results on reagent sheet.

Initials: RCV **Date:** 5/7/99

QC105 Alpha-Amylase Gel Radial Diffusion

Test Materials

Amylase Gel Buffer

Samples

Alpha-Amylase Standards
Human Saliva Stain
Deionized Water Negative Control

Procedure

Prepare a set of alpha-amylase standards containing 20 units of amylase activity/8ul, 2 units/8ul, 0.2units/8ul, 0.02 units/8ul, and 0.002 units/8ul in deionized water.

Extract a 5x5mm section of human saliva stain in deionized water for about 30 minutes. From an aliquot of this extract, prepare a 1/10 dilution in deionized water.

Test 8ul of each standard, the neat and 1/10 diluted saliva stain extracts, and a deionized water negative control as per the Amylase Gel Diffusion Method specified in the Forensic Biochemistry Manual.

Prepare a standard curve of the units of amylase activity (expressed logarithmically on x axis) versus the diameter of the diffusion circles around standard sample wells in the developed diffusion gel (plotted on y axis).

Determine amylase activity of the neat and 1/10 diluted saliva stain extract from the standard curve after measuring the diameter of the diffusion circle around both sample wells.

Specifications

The diameter of the clear circles around standard wells needs to be linear with respect to the amylase activity expressed logarithmically.

The diameter of the clear circle around each sample well needs to fall between the lowest and highest points on the standard curve.

The calculated amylase activity of the neat and 1/10 diluted saliva stain extract should differ approximately by the factor of 10 and both should fall on each side of an adjacent point on the standard curve.

Documentation

Write the test results on the reagent sheet.

Attach appropriate worksheet to the reagent sheet.

Initials: RCl **Date:** 5/7/99

QC110 Amplification Kits

Test Materials

Components of AmpF1STR Blue, Green, Cofiler and Profiler Plus Kits to include the following:
- AmpF1STR Reaction Mix
- Positive Control
- Primer Mix
- Allelic Ladder

Samples

Two whole blood or stain samples of known type
One amplification negative
One positive control sample from the PCR typing kit

Procedure

Amplify the samples and a positive control from the kit according to the amplification protocol. No extract is added to the amplification negative.

Separate the amplification products on a gel or capillary electrophoresis instrument following the appropriate protocol in the Forensic STR Analysis Manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative and positive control must show no evidence of contamination.

Documentation

Write the test up on appropriate amplification and electrophoresis worksheets.

Attach the completed worksheets to the Kit Control Log (F160).

File the Kit Control Log and the worksheets together in the appropriate QC reagent binder.

Initials: RC **Date:** 5/7/99

QC115 Autoclaving

GLASSWARE/EQUIPMENT

All glassware must be clean and dry prior to autoclaving (refer to QC175 for standard glassware cleaning procedure).

Cover glassware openings with aluminum foil.

Attach a strip of autoclave time tape to the aluminum foil on each piece.

Bottles should be loosely capped.

Small items may be autoclaved inside a beaker covered with foil.

SOLUTIONS

Falcon polypropylene conical tubes and glass bottles should be loosely capped. Small tubes are autoclaved inside a beaker.

Attach a strip of autoclave time tape to the object being autoclaved.

Do not fill bottles and tubes more than 75% of capacity.

OPERATION

The drain should be closed. The chamber should be filled with deionized water to the fill line (approximately 4 L). Load the chamber and close the door. Select exhaust, temperature and set the timer. Use fast exhaust for glassware and equipment and slow exhaust for solutions. The autoclave starts automatically and should not be opened until all of the pressure is released. If additional autoclaving is needed, refill water chamber and repeat procedure.

MAINTENANCE

Once all autoclaving has been done, the chamber should be drained of water by opening the drain knob and the door should be left open.

Initials: RCJ **Date:** 5/7/99

QC130 Capillary Electrophoresis (ABI 310)

Test Materials:

50μm Capillary
Performance Optimized Polymer 4
310 Genetic Analyzer Buffer with EDTA
Formamide (Deionized)
CXR Size Standard

Samples

The QC test can be performed using either the Cofiler, AmpflSTR Blue or Green allelic ladder, and amplified products.

Run amplified products from two known DNA samples at all blue or green loci; an allelic ladder, amplified positive control DNA, and a reagent blank, where no amplified product is added.

Procedure

Electrophorese samples according to the capillary electrophoresis protocol.

Analyze samples according to the Genescan Analysis and Genotyper protocols as described in the Forensic STR Analysis Manual.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of DNA.

Documentation

Write up the test on appropriate capillary electrophoresis run worksheets.

Attach the completed worksheets to a Raw Materials Log Sheet (F183).

File reagent sheet and CE run worksheets together in the appropriate QC reagent binder.

Initials: RCS **Date:** 5/7/99

QC140 Centrifuge Cleaning

Centrifuges are cleaned with a 10% bleach solution on a monthly basis. This insures that the centrifuge surface will be relatively clean of DNA that may have built up through normal laboratory use.

Both the inside chamber, rotor, and outside of the centrifuge should be wiped with the 10% bleach solution. This first wipe is then followed by another wipe, now using 70% ethanol. The ethanol is used to clean the surfaces from bleach and to complete the decontamination/disinfection process.

Cleaning of centrifuges is recorded on a Maintenance Log Sheet (F165) and filed in the Centrifuge Maintenance Log Binder.

Initials: RCS **Date:** 5/7/99

QC145 Chelex Extraction

Test Materials

Chelex, 5%

Samples

Two whole blood or stain samples of known type
One negative control sample

Procedure

Extract the two known samples and the negative control sample according to the Chelex extraction procedure for whole blood and bloodstains as described in the Protocols for Forensic STR Analysis Manual.

Amplify the samples according to the appropriate amplification protocol.

Hybridize or electrophorese the samples according to the appropriate protocol.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The negative control sample must show no evidence of contamination.

Documentation

Fill out the appropriate worksheets.

Attach the completed worksheets to the appropriate reagent sheet.

File the reagent sheet and the worksheets in the appropriate QC reagent binder.

Initials: RCJ **Date:** 5/7/99

QC150 Christmas Tree Stain for Spermatazoa

Test Materials:

Nuclear Fast Red
Picric Indigo Carmine

Samples

One positive control sperm sample heat fixed to a slide.

Procedure

Apply the Nuclear Fast Red and Picric Indigo Carmine to the cells and view the slide as described in the Forensic Biochemistry Methods Manual.

Specifications

There should be a visible acrosome and nucleus stained red.
The tail should be stained green.

Documentation

The slide should be enclosed in a slide mailer with all pertinent information listed on the front, encased in a plastic Kapak bag and attached to the appropriate reagent sheet.

Initials: RCS **Date:** 5/7/99

QC155 Clean Run

This procedure is used to pinpoint sources of contamination when a typing problem arises.

Samples

two whole blood or bloodstain samples of known type
one extraction negative
one amplification negative
one electrophoresis negative
one positive control sample from the DNA typing kit (if applicable)

Procedure

Extract the control samples and the extraction negative according to the Chelex extraction procedure for whole blood and bloodstains as described in the Protocols for Forensic STR Analysis Manual. The extraction negative control is a reagent control containing deionized water in place of sample. This sample should be handled the same way as the other samples, but no substrate is added.

Amplify the samples with the positive control from the kit (if applicable) and an amplification negative according to the appropriate amplification protocol. No Chelex extract is added to the amplification negative. This negative is used to evaluate contamination from the reagents and equipment in the amplification area.

Electrophorese the samples with an electrophoresis negative control, according to the appropriate protocol. No amplified or chelex extract is added to the electrophoresis or quantiblot negative controls, respectively.

Evaluation

If only the extraction negative shows contamination, the problem has occurred during the extraction step.

If the amplification negative shows contamination while the amplification negative is clean, the problem has occurred during the amplification set-up.

If only the positive controls appear contaminated, the problem might be a contaminated positive control.

Initials: RG **Date:** 5/7/15

QC155 Clean Run

Individual clean runs have to be evaluated on a case by case basis. It may be useful to determine what components have been changed since the last successful typing and to work from there.

Documentation

Write the clean run up on a set of appropriate worksheets.

Initials: RCA **Date:** 5/17/99

QC160 Differential Extraction

Test Materials

Chelex, 20%

Samples

One swab with epithelial and sperm cells of known type.
One negative control sample.
One positive control sample from the DNA typing kit (if applicable).

Procedure

Extract the known swab and the negative control sample according to the differential extraction procedure in the forensic DNA manual.

Amplify the samples and a positive control from the kit according to the appropriate amplification protocol.

Electrophorese the samples according to the appropriate protocol.

Specifications

Each sample fraction must match the assigned type within the current interpretation guidelines.

The negative control sample must show no evidence of contamination.

Documentation

Write the test up on a set of appropriate worksheets.

Attach the completed worksheets to the Solution Sheet.

File solution sheet and worksheets in the appropriate QC reagent binder.

Initials: RCS **Date:** 5/17/99

QC165 Gel Electrophoresis (ABI377)

Test Materials:

Ammonium Persulfate
Formamide
Formamide + Loading Buffer (5:1)
GS500 ROX
Long Ranger
Sequencing Loading Buffer
Temed
Urea

Samples

Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample

Procedure

Amplify the samples and a positive control using the appropriate reaction mixture according to the amplification protocol. No extract is added to the amplification negative.

Electrophorese samples according to the gel electrophoresis methods protocol.

Analyze samples according to the STR Gel Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on appropriate amplification and STR gel worksheets.

Attach the completed worksheets to the appropriate reagent sheet or raw material log sheet (F183).

File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.

Initials: RCJ **Date:** 5/7/99

QC170 Gel Electrophoresis (Yield Gel)

Test Materials

Lambda Marker
Yield Calibrators
Calibration Control

Procedure

Prepare a yield gel (substituting 2.0 g agarose/ 200 mL) according to the protocol in the STR Manual.

The test material and standards should be heated to 65°C and centrifuged as specified in the STR Manual.

Load the gel.

For quality control of Yield Calibrators, the previous lot of yield calibrators should also be electrophoresed as specified above.

Electrophorese and photograph as specified for a yield gel.

Specifications

Lambda Marker-	The photograph should display the banding pattern specified by the manufacturer
Yield Calibrators-	From the photograph, the new lot should have comparable intensities to the old lot. Each calibrator should have the correct relative intensity compared to the other calibrators. Each calibrator should appear as a single band with no trailing, at or above the highest band of the lambda standard. The calibration control should quanitate correctly.

Calibration Control- From the photograph, the calibration control should appear as a single band with no trailing, at or above the highest band of the lambda standard. The calibration control should quanitate correctly.

Documentation

Write the test up on the appropriate worksheets.

Attach the completed worksheet to the appropriate reagent sheet.

File the solution sheets and the worksheets in the appropriate QC reagent binder.

Initials: RCJ **Date:** 5/7/99

QC175 Glassware Cleaning

General Procedure

Most pieces of laboratory glassware can be cleaned by washing and brushing with a solution of detergent. Detergent is available from the OCME stockroom.

Rinse each piece at least three times with tap water to remove all detergent residue.

Rinse each piece several times with deionized water. If the surface is clean, the water will wet the surface uniformly. On soiled glass the water stands in droplets. If spotting is observed during the deionized water rinse, the detergent wash should be repeated. If spotting is observed after a second detergent wash, an acid rinse may be necessary (see below).

Allow the glassware to dry at room temperature on a drying rack.

Dishwasher

Load the dishwasher with glassware and put a scoop (approximately 42 g) of non-foaming, laboratory dishwasher detergent in the detergent cup. **Do not use regular laboratory detergent!**

Turn on the dishwasher using the steam scrubbing cycle. When the cycle is finished, remove the clean glassware.

Alternative Cleaning Procedures

When glassware cannot be completely cleaned by scrubbing with a detergent solution, other cleaning methods must be used.

Agarose
Solidified agarose in flasks can be redissolved by adding water to the flask and heating in the microwave. Solidified agarose in graduated cylinders can be removed with a brush. It is best not to use boiling water to redissolve solidified agarose in graduated cylinders, since this may affect the calibration of the cylinder over time.

Acid Rinse
Stubborn films and residues which adhere to the inside of flasks and bottles may often be removed by rinsing with dilute acetic or nitric acid. Some glassware may need to soak in dilute acid overnight. Any acid rinse must be followed by multiple rinses with deionized water to remove any acid residue.

Initials: **Date:**

QC180 Isoelectric Focusing: **Erythrocyte Acid Phosphatase (ACP)**

Test Materials:

ACP Isoelectric Focusing Plates
Anode solution
Cathode solution
ACP reaction buffer
ACP standards (BA, B, A, and C and R containing phenotypes)
Methylumbelliferyl phosphate
0.05 M DTT

Samples

Use two blood samples of known types for positive controls.
Use 0.05 M DTT for negative control.

Procedure

Bloodstains and/or commercially obtained samples containing ACP BA phenotype are to be tested as per the ACP by IEF method specified in the Biochemistry Methods Manual.

The tested extract is to be run in triplicate with varying volume size (15uL, 10uL, and 5uL). Ten microliters of the negative control is also tested.

Specifications

B1, B2, and A bands must be visible and sharply defined in at least one sample volume. The volume giving optimal banding will be used in casework.

Band separation must be as follows:

Bands	Allowable Separation
B1 to B2	≥8mm
B2 to A	≥10mm
A to Hb	≥1mm

Documentation

Write the test up and attach photographic documentation to appropriate test worksheets.

Attach worksheet to reagent worksheet.

Initials: RC **Date:** 5/7/99

QC185 Isoelectric Focusing : Esterase D (ESD)

Test Materials:

ESD Isoelecric Focusing Plates
Anode Solution
Cathode Solution
ESD Reaction Buffer
ESD Standards (1, 2-1, and 5-1)
Methylumbelliferyl acetate

Samples

Use two blood samples of known types for positive controls.
Use 0.05 M DTT for negative control.

Procedure

Bloodstains and/or commercially obtained samples containing ESD 1, 2-1, and 5-1 phenotypes are to be tested as per the ESD by IEF method specified in the Biochemistry Methods Manual.

The tested extracts are to be run in triplicate with varying volume size (15uL, 10uL, and 5uL). Ten microliters of the negative control is also tested.

Specifications

In order for ESD IEF plates to be deemed acceptable for casework, the following is the allowable separation for adjacent bands on ESD phenotypes:

ESD Type	Bands	Allowable Separation
1	top-bottom	≥3mm
2-1	top-middle	≥1mm
	middle-bottom	≥1mm
5-1	top-middle	≥3mm
	middle-bottom	≥3mm

In order for ESD standards to be deemed acceptable for casework, clearly typeable results must be observed with all sample volumes tested.

Initials: RC **Date:** 5/7/99

QC190 Isoelectric Focusing: Hemoglobin

Test Materials:

Hemoglobin Isoelectric Focusing Plates
Anode Solution (1% Acetic Acid)
Cathode Solution (1% Ethanolamine)
0.05% Potassium Cyanide
pH 3-10, 4-6, 6-8 Ampholyte
AFSC Standard

Samples

AFSC Standard
Potassium Cyanide

Procedure

Dilute 5ul of the AFSC hemoglobin control with 45ul 0.05% potassium cyanide.

Fifteen microliter (15ul), 10ul, and 5ul aliquots of the diluted standard is tested as per the hemoglobin IEF method as specified in the Forensic Biochemistry Methods Manual. Ten microliters of potassium cyanide is also tested.

Specification

All four bands must be visible and sharply defined in at least one standard. The volume giving optimal banding will be used in casework.

Band separation must be as follows:

Bands	Allowable Separation
A to F	>2mm
F to S	>3mm
S to C	>6mm

Documentation

Write the test up and attach photographic documentation to appropriate test worksheets.

Attach worksheet to reagent worksheet.

Initials: RD **Date:** 5/2/99

QC195 Isoelectric Focusing: Phosphoglucomutase (PGM)

Test Materials:

PGM Isoelectric Focusing Plates
Anode solution
Cathode solution
PGM reaction buffer
PGM standards (2+2-1+1- containing phenotypes)

Samples

Use two blood samples of known types for positive controls.
Use deionized water for negative control.

Procedure

Bloodstains and/or commercially obtained samples containing PGM phenotype are to be tested as per the PGM by IEF method specified in the Biochemistry Methods Manual.

The tested extract is to be run in triplicate with varying volume size (15uL, 10uL, and 5uL). Ten microliters of the negative control is also tested.

Specifications

2+, 2-, 1+, and 1- bands must be visible and sharply defined in at least one sample volume. The volume giving optimal banding will be used in casework.

Band separation must be as follows:

Bands	Allowable Separation
type 2+2-	> 4 mm
type 2-1+	> 6 mm
type 1+1-	> 2 mm

Documentation

Write the test up and attach photographic documentation to appropriate test worksheets.

Attach worksheet to reagent worksheet.

Initials: RCJ **Date:** 5/7/99

QC200 Kastle -Meyer Presumptive Test for Blood

Test Materials

Kastle-Meyer Reagent

Samples

Whole Blood
Deionized Water Negative Control

Procedure

Prepare ten-fold serial dilutions of whole blood in deionized water beginning with 1/10 and ending with a 1/1,000,000 dilution.

Place one drop of each dilution on a strip of filter paper (including a neat sample) and deionized water and allow to dry.

Test each dried drop with Kastle-Meyer reagent as per the Forensic Biochemistry manual.

Specifications

Reagent sensitivity must not be less than 1/1000 dilution of whole blood.

The deionized water must give a negative result.

Positive reactions must be observed in any dilution only after the addition of 3% hydrogen peroxide.

Documentation

Write test results on Reagent Sheet.

Initials: RCJ **Date:** 5/7/95

QC205 Leucomalachite Green Presumptive Test for Blood

Test Materials

Leucomalachite Green Reagent

Samples

Whole Blood
Deionized Water Negative Control

Procedure

Prepare ten-fold serial dilutions of whole blood in deionized water beginning with 1/10 and ending with a 1/1,000,000 dilution.

Place one drop of each dilution on a strip of filter paper (including a neat sample) and deionized water and allow to dry.

Test each dried drop with Leucomalachite Green reagent as per the Forensic Biochemistry manual.

Specifications

Reagent sensitivity must not be less than 1/1000 dilution of whole blood.

The deionized water must give a negative result.

Positive reactions must be observed in any dilution only after the addition of 3% hydrogen peroxide.

Documentation

Write test results on Reagent Sheet.

Initials: RG **Date:** 5/7/99

QC210 Matrix File

Making a matrix

Introduction

A matrix file is required by the 310 and 377 fluorescent fragment detection software in order to subtract overlapping wavelength components from the different color signals. Therefore the matrix consists of a table of numbers that quantitatively reflect the amount of each dye detected in each color filter.

The necessity to make a new matrix file arises from any changes that may have occurred to the optical properties of an instrument; this might be a repair or replacement of a component of the optical system or a change in the gel composition. Since there a subtle differences between the different instruments each instrument has to have its own matrix file and gels or runs performed have to be analyzed with the matrix belonging to the instrument that was used. Also, the matrix has to be made for each different series of dyes that are used on an instrument.

Also due to minor shifts in the quality of the CCD camera, the laser, the glass plates, or the reagents it can become necessary to make a new matrix, even though no changes were made. The following occurrences are indications that the old matrix does not achieve the correct amount of spectral overlap:

- pull up peaks underneath peaks of a height less than 2000fu
- pull down events in a different color caused by peaks in another color
- elevated baseline of a different color between two peaks in another color

The matrix file is made by running the pure dyes and then performing the Genscan software step "New Matrix" that is described below. Different labeling chemistries of course require different matrices to be used during the analysis.

The table below shows the different labels used for fluorescent system employed by the Department of Forensic Biology for casework and research. The table also displays how the matrix standards are supplied by either Perkin Elmer of Promega, and which virtual filterwheel on the instrument corresponds to which dye.

When making a new matrix **select the appropriate four samples for each system**. Standards for different systems can be run together. The matrix standards have to be run under the regular conditions, but with no matrix applied to the run. Matrix standards can be coloaded with other samples, which can be analyzed separately afterwards.

Initials: RCJ **Date:** 5/7/99

QC210 Matrix File

Table 1: Available Matrix Standards

Multiplex systems	Color	Label	Contained in PE kit	Filterwheel required
QUAD, YM1	Blue	6-FAM	Fluorescent Amidite Matrix Standard Kit	A
	Green	JOE	Dye Primer Matrix Standards	
	Yellow	NED	NED Matrix Standard	
	Red	ROX	Dye Primer Matrix Standards	
AmpFlSTR Blue, Green, Cofiler, Profiler Plus	Blue	5-FAM	Dye Primer Matrix Standards	A or F
	Green	JOE	Dye Primer Matrix Standards	
	Yellow	NED	NED Matrix Standard	
	Red	ROX	Dye Primer Matrix Standards	
Powerplex systems	Blue	Fluorescein	Promega Powerplex kit	A
	Green	HEX	Fluorescent Amidite Matrix Standard Kit	
	Yellow	TMR	Promega Powerplex kit	
	Red	ROX	CXR standard from Promega Powerplex kit	
dRhodamine Sequencing Big Dye Sequencing	Dye primer C	dR110	dRhodamine Matirx Standards	E
	Dye primer A	dR6G	dRhodamine Matirx Standards	
	Dye primer G	dTAMRA	dRhodamine Matirx Standards	
	Dye primer T	dROX	dRhodamine Matirx Standards	

NOTE: Matrix standards have to be mixed with formamide and denatured, but **DO NOT** add the red size standard.

1.) For 310 Mix 1μL of each matrix standard with 12μL of deionized formamide only. Denature at 95°C for three minutes, then chill on ice and place in the 48-well sample tray. **Do two injections each.**

2.) For 377 Mix 4μL of each matrix standard with 4μL of blue formamide only. Denature at 95°C for two minutes, then chill on ice before loading. Load twice, 3μL each..

Don't forget to load both 5-FAM and 6-FAM when making a STR matrix.

Electrophoresis and Making a Matrix file

1.) For 310 Set up sample sheet, injection list as usual (see STR Manual). The only modification is that in the injection list under Matrix file you have to select "none". Prepare the samples a stated above and start the run.

The duplicates of the standards are only meant as backup. It is not necessary to use both sets. For each standard select the more intense one of the duplicates.

After the run is complete the Genescan analysis software should be open already. Under **File** select **New** and there select **Matrix** .

In the window that appears indicate the sample file that corresponds to each dye color. Refer to **Table 1** for which color has which name and in order to decide which colors to combine for each systems. It may be necessary to browse and open the run folder. Select starting scan numbers of 3300 for each sample. This starting number is intended to exclude the primer peaks.

Under points enter 2500 and click O.K. The computer makes the matrix and the following window appears:

NED POP 4

Reactions

	B	G	Y	R
B	1.0000	0.6102	0.0397	0.0022
G	0.6082	1.0000	0.4699	0.0076
Y	0.3938	0.7060	1.0000	0.1063
R	0.1821	0.3768	0.5563	1.0000

Under **File** select **Save**. Save the new matrix twice: once in the GS Matrix folder in the Genescan analysis folder, and **IMPORTANT** in the ABI folder in the Macintosh System folder. In order to save a copy in each of these folders, highlight the icon after it has been saved once, under **File** select **Duplicate**. Then drag one of the copies in the other folder. Only if the matrix is saved in the system folder it will be available as an option in the injection list.

As a filename use the instrument name and the creation date:
e.g. CE3 4/99

Proceed with the section **Quality Control Testing of Genescan Matrix Files** in order to test the new matrix and print out the documentation.

If runs are analyzed on separate terminals the matrix for the different instruments have to be made available. Copy the file in the GS Matrix folder in Genescan folder on the hard drive.

2.) For 377 Genescan

Set up the gel and the electrophoresis conditions as usual (see STR Manual). The only modification is that under Matrix file you have to select "none".

Load 3μL each twice. Avoid spillover. If possible leave an empty lane between the standards.

The duplicates of the standards are only meant as backup. It is not necessary to use both sets. For each standard select the more intense one of the duplicates.

After the gel run, open **Genescan analysis**, open the gel file, select a gel range starting at about 1500, fill out the sample sheet and extract the lanes as usual. At this point you will see the Analysis Control Project window.

Under **File** select **New** and there select **Matrix** .

In the window that appears indicate the sample file that corresponds to each dye color. Refer to **Table 1** for which color has which name and in order to decide which colors to combine for each systems. **ATTENTION**: use 6-FAM once with all three other colors, then repeat using 5-FAM and all three other colors. It may be necessary to browse and open the run folder. Select starting scan numbers that correspond with the above selected analysis range for each sample. This starting number is intended to exclude the primer peaks.

Under value enter 2500 points and click O.K. The computer makes the matrix and a

window as shown above appears.

Under **File** select **Save**. Save the new matrix twice: once in the GS Matrix folder in the Genescan analysis folder, and **IMPORTANT** in the ABI folder in the Macintosh System folder. In order to save a copy in each of these folders, highlight the icon after it has been saved once, under **File** select **Duplicate**. Then drag one of the copies in the other folder. Only if the matrix is saved in the system folder it will be available as an option in the injection list.

As a filename use the instrument name, the FAM used and the creation date:

e.g. Jeffreys 6-FAM 4/99

Repeat the making of the new matrix for the second blue color.

Proceed with the section **Quality Control Testing of Genescan Matrix Files** in order to test the new matrix and print out the documentation.

If runs are analyzed on separate terminals the matrix for the different instruments have to be made available. Copy the file in the GS Matrix folder in Genescan folder on the hard drive

3.) For 377 dRhodamine and Big Dye sequencing

Set up the gel and the electrophoresis conditions as usual. The only modification is that under Matrix file you have to select "none".

Load 3μL each twice. Avoid spillover. If possible leave an empty lane between the standards.

After the gel run, under **Sequence Analysis** open the gel file, select the gel range to exclude the primer peaks, fill out the sample sheet and extract the lanes as usual.

Open the **Data utility** application and from the **Utilities** menu select **Make Matrix** .

For a sequencing matrix each matrix standard has to be selected in different boxes three times. Follow the instructions below. As the starting scan number, select a the number that corresponds with the above selected analysis range for each sample. This starting number is intended to exclude the primer peaks.

A. Make the Dye Primer Matrix
Select each box and click on the sample file corresponding to the standards below:

C ... dR110
A ... dR6G

G... dTAMRA
T... dROX

Click **New File**. Name the file dRhod and save it in the **ABI folder** within the **System** folder
Click the Dye Primer Matrix radial button. Click O.K.

B. Make the Taq Terminator Matrix:
From the **Utilities** menu select **Make Matrix** .
Select each box and click on the sample file corresponding to the standards below:

C ... dROX
A... dR6G
G... dR110
T... dTAMRA

Click **Update File**. Choose dRhod and save it in the **ABI folder** within the **System** folder
Click the Taq Terminator Matrix radial button. Click O.K.

C. Make the T7 Terminator Matrix:
From the **Utilities** menu select **Make Matrix** .
Select each box and click on the sample file corresponding to the standards below:

C ... dR6G
A... dTAMRA
G... dROX
T... dR110

Click **Update File**. Choose dRhod and save it in the **ABI folder** within the **System** folder
Click the T7 Terminator Matrix radial button. Click O.K.

To check the matrix file, select **Copy Matrix** from the **Utilities** menu. Under source select **Instrument File** and choose **dRhod** form the **ABI folder** within the **System** folder. The matrix will be displayed on the screen, all three boxes should be filled, the corresponding numbers for each of the three boxes will be the same. Click **Cancel**.

NOTE: Not all three matrices are necessary for sequencing analysis, but the are necessary for terminator reactions sequencing data collection. The run will not start if only a terminator matrix is present. The error message that will appear if the primer matrix is missing will read "Taq is not found".

If sequencing runs are analyzed on separate terminals the make sure that you use the

correct matrix for the different instruments. If necessary, copy the file in the Sequencing Analysis folder on the hard drive

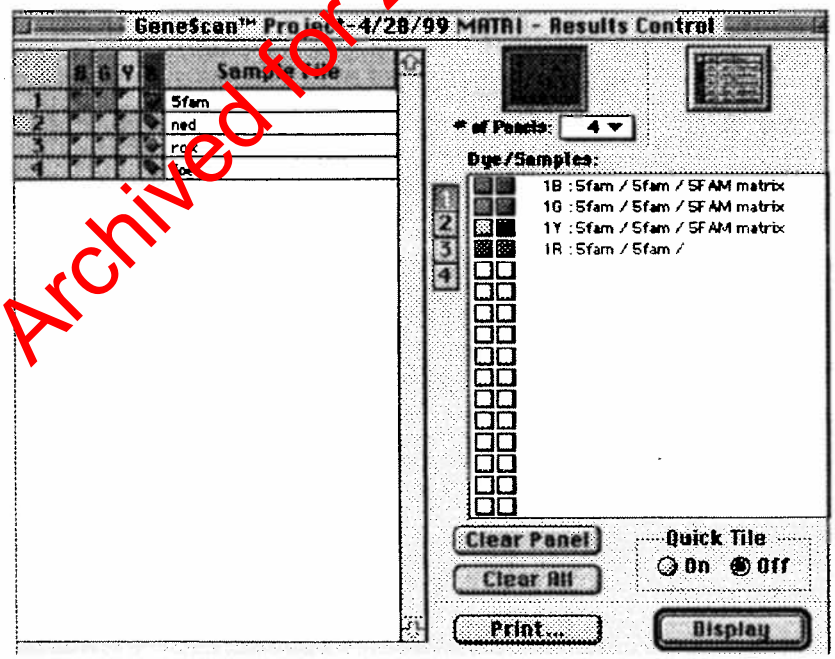
Quality control testing of Genescan STR matrix files

In order to test, if the new matrix is working correctly, it should be applied to the matrix standard sample files.

Open the project with the extracted matrix standards. Under **Samples** choose **Install new matrix**. Install the matrix you just made.

Click on the top blue, green, yellow, and red boxes to select the all colors for the analysis for all lanes. Click on the **Analyze** button in the upper left corner. All selected samples will be analyzed. There will be an error message in the analysis log window because the samples do not have a size standard. Ignore this message.

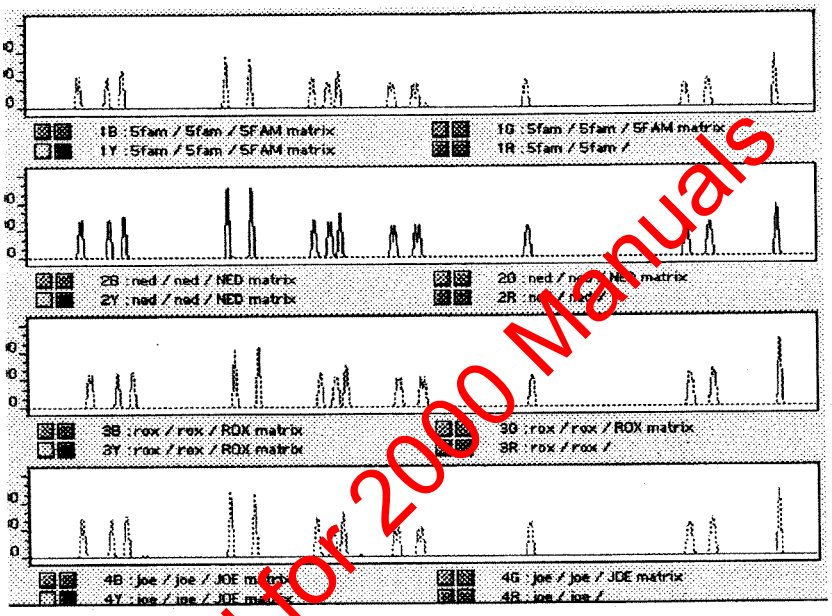
Open the results control window.



In the upper right hand corner, deselect the **Display Table** option by clicking on the icon, so that it is not indented anymore. Also switch **Quick Tile** to **Off**.

Display all colors in sample one in field one, sample two in filed two, and so on...

If the matrix is correct no pull-up peaks should be visible, all colors should only consist of one color. See example below.



Print out the following documentation for the **Matrix Log Book**:

For STRs: the Matrix number box (double click on the icon in the Matrix Folder in Genescan analysis folder to open the file and select print) , the electropherogram of the analyzed matrix standards (see above).

For Sequencing: the three Matrix number boxes

File these sheets together with the run control or gel sheets in the Matrix Log book.

Initials: RCJ **Date:** 5/7/99

QC220 Ouchterlony Radial Diffusion: Species Determination

Test Materials:

Tank Buffer
1% Agarose Gel

Samples

One serum sample positive control.
One corresponding α-serum sample.
One negative control (deionized water or saline).

Procedure

Prepare the tank buffer and agarose gel as described in the Quality Manual.
Punch holes in the solidified gel, load samples and develop gel as described in the Forensic Biochemistry Methods Manual.

Specifications

The positive control must give a positive result.
The negative control must give a negative result.

Documentation

Write the test up on an Ouchterlony Test Worksheet and attach it to the appropriate reagent sheet.

Initials: RCJ **Date:** 5/7/99

QC225 P30 ELISA

Test Materials

P30 Antigen
Monoclonal Anti-human P30
Polyclonal Anti-human P30
Alkaline Phosphatase Conjugate
IgG1, Kappa Chain (MOPC 21)
p-Nitrophenol Phosphate Tablets
Alkaline Substrate Buffer
PBS-BSA Solution
Phosphate Buffered Saline
Casein Stock Solution

Procedure - Monoclonal Anti-human P30 QC

Prepare 1/5,000 - 1/10,000 dilutions of monoclonal anti-human P30 with phosphate buffered saline.

Set up a microtiter plate as diagramed and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

Initials: RCJ **Date:** 5/7/98

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3-5, C-D:	1/5,000	monoclonal anti-human P30
6-8, C-D:	1/6,000	monoclonal anti-human P30
9-11, C-D:	1/7,000	monoclonal anti-human P30
3-5, G-H:	1/8,000	monoclonal anti-human P30
6-8, G-H:	1/9,000	monoclonal anti-human P30
9-11, G-H:	1/10,000	monoclonal anti-human P30

Note: 2-12, A-B and E-F are coated with 1/8000 MOPC as described in the Biochemistry Methods Manual.

Specifications

Determine the weakest dilution of antisera which gives a result for the 2ng P30 standard. Choose as the working titer the next strongest dilution.

Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation

Document test on a P30 ELISA worksheet.

Fill out a P30 Antisera and Reagents QC sheet (including working titer)..

Attach P30 ELISA worksheet to QC sheet.

Procedure - Polyclonal Anti-human P30 QC

Prepare 1/500 - 1/3000 dilutions of polyclonal anti-human P30 with phosphate buffered saline.

Set up a microtiter plate as diagramed and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

Initials: RG **Date:** 5/7/99

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3-5, C-D: 1/500 polyclonal anti-human P30
6-8, C-D: 1/1,000 polyclonal anti-human P30
9-11, C-D: 1/1,500 polyclonal anti-human P30
3-5, G-H: 1/2,000 polyclonal anti-human P30
6-8, G-H: 1/2,500 polyclonal anti-human P30
9-11, G-H: 1/3,000 polyclonal anti-human P30

Note: 2-12, A-B and E-F are coated with 1/8000 MOPC as described in the Biochemistry Methods Manual.

Specifications

Determine the weakest dilution of antisera which gives a result for the 2ng P30 standard. Choose as the working titer the next strongest dilution.

Once the proper working titer has been established, also perform specificity procedure (see below).

Documentation

Document test on a P30 ELISA worksheet.

Fill out a P30 Antisera and Reagents QC sheet (including working titer) .

Attach P30 ELISA worksheet to QC sheet.

Initials: RCJ **Date:** 5/7/15

Procedure - Alkaline Phosphatase Conjugate QC

Prepare 1/500 - 1/3,000 dilutions of alkaline phosphatase conjugate with phosphate buffered saline.

Set up a microtiter plate as diagramed and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
B	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
C	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
D	PBS	W	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	6ng	
E	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
F	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
G	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	
H	PBS	W	6ng	2ng	10ng	6ng	2ng	10ng	6ng	2ng	10ng	

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of P30 antigen

3-5, C-D: 1/500 alkaline phosphatase conjugate
6-8, C-D: 1/1,000 alkaline phosphatase conjugate
9-11, C-D: 1/1,500 alkaline phosphatase conjugate
3-5, G-H: 1/2,000 alkaline phosphatase conjugate
6-8, G-H: 1/2,500 alkaline phosphatase conjugate
9-11, G-H: 1/3,000 alkaline phosphatase conjugate

Note: 2-12, A-B and E-F are coated with 1/8000 MOPC as described in the Biochemistry Methods Manual.

Specifications

Determine the weakest dilution of alkaline phosphatase conjugate which gives a result for the 2ng P30 standard. Choose as the working titer the next strongest dilution.

Once the proper working titer has been established, also perform specificity procedure (see below).

Initials: RCJ **Date:** 5/7/99

Documentation

Document test on a P30 ELISA worksheet.

Fill out a P30 Antisera and Reagents QC sheet (including working titer).

Attach P30 ELISA worksheet to QC sheet.

Specificity Procedure - All Other Reagents

Prepare 1/25 - 1/25,000 serial dilutions (using 10-fold dilution steps) of stains prepared from semen, blood, urine, and saliva from healthy males.

Set up a microtiter plate as diagramed and perform P30 ELISA as specified in the Forensic Biochemistry Methods Manual.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	sem	sem	b	b	u	u	sal	sal
B	PBS	W	2ng	10ng	sem	sem	b	b	u	u	sal	sal
C	PBS	W	2ng	10ng	sem	sem	b	b	u	u	sal	sal
D	PBS	W	2ng	10ng	sem	sem	b	b	u	u	sal	sal
E	PBS	W	6ng		sem	sem	b	b	u	u	sal	sal
F	PBS	W	6ng		sem	sem	b	b	u	u	sal	sal
G	PBS	W	6ng		sem	sem	b	b	u	u	sal	sal
H	PBS	W	6ng		sem	sem	b	b	u	u	sal	sal

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
2ng, 6ng, 10ng - quantity of standard P30 antigen

5A-H, 6A-H: semen stain (sem), 1/25 - 1/25,000 dilution
7A-H, 8A-H: blood stain (b), 1/25 - 1/25,000 dilution
9A-H, 10A-H: urine stain (u), 1/25 - 1/25,000 dilution
11A-H, 12A-H: saliva stain (sal), 1/25 - 1/25,000 dilution

Initials: RG **Date:** 5/7/99

Specifications

All samples of blood, urine, and saliva must give negative results.

Semen results must yield positive results with values indicative of serial dilutions.
P30 standard results must reflect standard quantities.

Documentation

Fill out and attach P30 ELISA worksheet to an appropriate reagent sheet or raw material log sheet(F183).

Initials: AJ **Date:** 5/7/99

QC240 PCR Amplification

Test Materials
Blue STR Reaction Mixture
BSA
dNTPs set
Cofiler STR Reaction Mixture
Green STR Reaction Mixture
$MgCl_2$
PCR Buffer
Primers
Profiler Plus Reaction Mixture
Quad STR Positive Control
Quad STR Reaction Mixture
Taq
Y STR Positive Control
Y STR Reaction Mix

Samples

Two whole blood or stain samples of known type.
One amplification negative.
One positive control sample

Procedure

Amplify the samples and a positive control using the appropriate reaction mixture according to the amplification protocol. No extract is added to the amplification negative.

Electrophorese samples according to the gel electrophoresis protocol.

Analyse samples according to the STR Gel Analysis and Genotyper Instructions protocols.

Specifications

Each sample must match the assigned type within the current interpretation guidelines.

The amplification negative must show no evidence of contamination.

Documentation

Write the test up on an appropriate a mplification and STR gel worksheets.

Attach the completed worksheets to the appropriate reagent sheet or raw material log sheet (F183).

File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.

Initials: RCJ **Date:** 5/7/99

QC250 QuantiBlot Hybridization

Test Materials

BSA, 5 mg/mL
Chromagen
dNTPs Set
Digest Buffer
DTT, 1 M
$MgCl_2$
PCR Buffer
Phosphate Buffered Saline (PBS)
Primers Used for Quad & Y STR Analysis
Proteinase-K Enzyme, 20 mg/ml
QuantiBlot DNA Standards
QuantiBlot Hybridization Solution
QuantiBlot Kits
Calibrators 1 & 2
DNA Probe
Enzyme Conjugate
QuantiBlot Spotting Solution
QuantiBlot Wash Solution
Sterile Water
Taq DNA Polymerase
TE^{-4}, 1X

Samples

Solution to be tested for the presence of DNA at the volume indicated in the QC section of the solution sheet. Test 20 µL of dNTP's set, 5 µL Taq, 25 µL PCR Buff II, 25 µL $MgCl_2$

Procedure

Hybridize the samples according to the Quantiblot protocol.

Specifications

Each QuantiBlot Calibrator must have an intensity bounded by the appropriate QuantiBlot DNA standard.
All of the QuantiBlot standards must be visible.
The tested solution must show no evidence of contamination. There must be no hybridization to the slot containing the tested solution.
The negative control must show no evidence of contamination.

Documentation

Write the test up on a QuantiBlot Hybridization Worksheet.
Attach the completed worksheet to the appropriate reagent sheet or raw material log sheet (F183).
File the reagent sheet or raw material log sheet and the worksheets in the appropriate QC reagent binder.
Note: Chromagen and components of the QuantiBlot Kits (with the exception of the QuantiBlot DNA Standards which are tested for each new lot) should be tested for each new vendor lot/ shipment.

Initials: RCJ **Date:** 5/7/09

QC255 Species Crossover Electrophoresis

Test Materials:

Tank Buffer
1% Agarose Gel

Samples

One positive control serum sample.
One corresponding α-serum sample.
One negative control (distilled water or saline).

Procedure

Prepare tank buffer and agarose gel as described in the Quality Manual; Appendix A.
Punch holes in solidified gel, load samples and develop gel as described in the Forensic Biochemistry Methods Manual.

Specifications

The positive control must give a positive result.
The negative control must give a negative result.

Documentation

Write the test up on Crossover Electrophoresis Worksheet and attach the completed sheet to the appropriate reagent sheet.

Initials: RG **Date:** 5/7/99

QC265 Takayama Hemoglobin Test

Test Materials:

Takayama Reagent

Samples

One positive control consisting of a whole blood or bloodstain sample.
One negative control consisting of saline or deionized water.

Procedures

Perform the Takayama test on the positive and negative controls as described in the Forensic Biochemistry Methods Manual.

Specifications

The positive control must give a positive result.
The negative control must give a negative result.

Documentation

The test should be documented on a Takayama reagent sheet.

Initials: KCJ **Date:** 5/17/05

QC305 Urea Gel Diffusion

Test Materials:

Urea test and blank diffusion plates

Samples

Urea standards
Dried urine stain

Procedure

Prepare urea standards containing 5g/100ml, 0.5g urea/100ml, 0.05g urea/100ml, and 0.005g urea/100ml respectively, in deionized water.

Extract a 1cmx1cm urine stain in 200ml deionized water and prepared a 1/10 dilution of the extract in deionized water.

Test each urea standard, the neat and 1/10 urine stain extract dilution, and a deionized water blank as per the urine gel diffusion procedure specified in the Biochemistry methods Manual.

Prepare a standard curve of urea concentration (expressed logarithmically on x axis) versus the adjusted diffusion radius (determined by subtracting the mean diffusion radius of each standard on the blank plate from the mean diffusion radius on the test plate).

Plot the adjusted diffusion radius of the neat and 1/10 diluted extracts of the known urine stain on the standard curve.

Specifications

The adjusted diffusion radius of the standard needs to be linear with respect to the urea concentration expressed logarithmically.

The adjusted diffusion radius of the neat and 1/10 diluted urine stain extracts needs to fall between the highest and lowest points on the standard curve.

The calculated urea concentration of the neat and 1/10 diluted urine stain extracts needs to differ by an approximate factor of 10.

Documentation
Write test results on the appropriate reagent sheet.

Attach appropriate worksheets to the reagent sheet.

Initials: RCJ **Date:** 5/7/99

QC120 Balances: Verification and Maintenance

Routine Weight Measurements

1. Press the control bar once to turn on the power. Allow the readout to stabilize to 0.000.
2. Place the weigh paper or weigh boat on the pan of the balance. Allow the readout to stabilize.
3. Press the control bar once to tare the balance.
4. Make the desired measurement.
5. When finished, pull the control bar up to turn off the power. Clean out the weighing chamber with the small brush or a damp paper towel, being careful not to disturb the pan.

Mettler AE260 Analytical Balance Two-point Calibration

A two-point standardization should be performed monthly using the protocol described below:

1. Press the control bar once to turn on the power. Allow the readout to stabilize to 0.000.
2. Close all the doors surrounding the weighing chamber.
3. Press and hold the control bar until the readout says CALIB. The balance is calibrating at zero grams.
4. When the readout flashes 100, slide the lever on the right side back to release the internal 100 gram standard weight. Allow the balance to calibrate at 100 grams.
5. When the readout flashes 0, slide the lever forward. Allow the readout to stabilize.

The balance is calibrated and ready for use.

Balance Four-point Weight Verification

Each week, the balance is verified using four standard weights.

1. Weigh the first standard. Record the standard weight and the measured weight on the Balance Verification and Maintenance Log (F100).
2. Repeat the measurements for the other three standard weights. Record all measurements.
3. File Balance Verification and Maintenance Logs into the Scale Log Binder.

Calibration and Maintenance

Balances should be calibrated yearly by an outside contractor.

Initials: RCJ **Date:** 5/7/99

QC125 Biological Safety Cabinet/Fume Hood: Operation and Maintenance **Page 1 of 2**

Routine Use

Turn the blower on and **WAIT** 15 minutes before using the hood. Leave the blower on while you are working in the hood.

Turn on the fluorescent light (NOT the UV light of the Biological Safety Cabinet).

Wipe all exposed hood surfaces with 70% ethanol. This must be done by every individual, each time they start to work in the hood.

Line the work surface with absorbent pads. Put the plastic side down and the paper side up. Do not block the vents.

Work on the absorbent pads following all of the safety precautions listed above.

In case of a spill onto the hood surface, decontaminate with 10% bleach for 10 minutes. Absorb the bleach onto a paper towel and rinse the surface with 70% ethanol.

NOTE: All the bleach must be rinsed from the hood surface with the ethanol. Otherwise the hood will corrode.

If the blower stops running, DISCONTINUE all work and safely seal up all samples. **The hood no longer offers any protection.**

When you are done working, discard the absorbent pads and change your top layer of gloves.

Wipe all exposed surfaces with 70% ethanol and then discard your gloves layer by layer in the red biohazard bags.

If using a Biological Safety Cabinet that is equipped with a UV light, turn the UV light on for 1 hour. Do not expose yourself to the UV.

Shut off the blower and UV (if applicable). Do NOT leave on overnight.

NOTE: Do not work with any organic solvents (except ethanol) in the biosafety hood. Use the Fume Hood for this purpose.

Initials: RCJ **Date:** 5/7/85

Procedure for Air Flow Measurements In Chemical Fume Hoods and Biological Safety Cabinets

Hood air flow measurements should be taken monthly and documented in the Chemical Fume Hood and Biological Cabinet Maintenance Log Binder. The Procedure is as follows:

1. Take Measurements using Tri-Sense Air Velocity Meter with air velocity probe.

2. Take measurements in the direct center of hood or cabinet working area.

3. Orientate open area of top of probe perpendicular to the base of working area.

4. Take measurements at 1inch, 6 inches, 12 inches, 18 inches, and 24 inches from base of working area. Record values (ft./min.) on the Hood Flow Rate Log (F155).

Maintenance

The hood is inspected by an outside vendor once a year. This information is also recorded in the Chemical Fume Hood and Biological Cabinet Maintenance Log Binder.

Initials: pcj Date: 5/7/99

There are two diagnostic tests run every month. The test results are recorded on a 310 Capillary Electrophoresis Diagnostic Log sheet (F105). These tests can be run while there is a capillary in the instrument. Make sure that the capillary is not damaged during the testing. Especially since the second test requires the removal of the capillary from the laser window. The first test cannot be run with the 310 Collection Software open!

LASERTEST

1.) Quit 310 Collection Software if necessary.

2.) To access the diagnostic test files, open the **310 diagnostics** folder located on the hard drive. And click on the 310 diagnostics icon. At this point you will receive a warning, that the 310 diagnostics software cannot run if the Prism collection software is already running. You can check this by going to the upper left hand corner, and clicking on the finder icon. If it is not running, click **Continue,** otherwise click **Quit** and start with step1).

At this point you may receive the message "Establishing serial communication link with 310 instrument. This may take several seconds. Do not click Abort!!! Afterwards you might get the message "Instrument is not responding. Wait 10 seconds and then click o.k." Do wait and click **o.k.**.

From the first menu of options choose **Test Components.** From the second menu of test components choose **Laser Power**.

3.) Click on **start**. The values for the laserpower mW and the laserpower Amps will appear on the screen, ignore the first two readings and record the 3rd, the 4th, and the 5th reading on log sheet F105.
Also record the pass or fail status.

4.) After the 5th set of values appeared, wait till the indicator on the left side shows 100% done, then click on **Done**. The message that will appear says results not logged. To the question "log now" click **no**.

5.) On the 310 components menu press **Return.**
On the main diagnostics menu press **Quit.**

If the laser fails readings 3-5 take the instrument out of service and call the PE/ABD technical service representative.

CCD CAMERA SENSITIVITY TEST

For this test the regular capillary is replaced with a sensitivity standard capillary and a mock run is performed. The capillary does not have to be taken out, it is sufficient to temporarily remove it from the CCD camera lens window.

1.) Open the 310 Collection Software.

2.) Under **file** select **new** then select **sequence sample sheet**. In the first row (A1) put one sample name e.g. CCD test. If there is no module and no matrix selected, import any of the existing possibilities. The sections have to be filled, but the files will not be applied and are just fake. Close the sample sheet and save it as e.g. CCD test.

3.) Under **file** select **new** then select **sequence run.** Import the sample sheet that was created under 2.). Select **Test CCD sensitivity** as run module. Deselect Autoanalyze if necessary.

4.) Open the 310 instrument door, open the heat plate cover door, and the laser window door. Be careful not to damage the regularly installed capillary during the next steps. Move the capillary out of the laser window notch and bend it out of the way so that the laser window door and the heat plate cover can be closed without damaging the capillary.

5.) Take the sensitivity standard capillary provided by ABD/PE (part # 401928) and place its window in front of the camera lens. The yellow tag should be on top. Carefully close the laser window door, the heat plate cover and the instrument door.

6.) Click on **Run**. Under **Window** open **Status** to observe the progress. The program will collect data for 5 min. Then a second data collection set for 2.5 min will start. An alert message "EP current is zero" will pop up, click **o.k.**. Data collection will continue.

7.) When the alert prompt " Remove capillary" appears, open the instrument door, open the heat plate cover and the laser window door and remove the sensitivity standard. Do not put the old capillary back yet!! Close all doors, click **o.k.**, the run will resume automatically. Data will be collected for 2.5 minutes. Click **o.k.** to the alert prompt that the EP current is zero.

8.) After the data collection is completed, close the run, save the injection list, and quit the data collection program.

9.) On the hard drive open the **310 diagnostics** folder and click on the **310 diagnostics icon**. From the main menu select **Analysis**. From the Analysis menu select **Signal to Noise Auto**.

10.)Click on **Start**. Import the mock run from before, which should be in the current run folder. Highlight the sample file and click ok. The data will be analyzed automatically.
Record the relevant values on form F037B, the relevant values are 586 S/N ratio, 625 S/N ratio, 586 noise w/cap, 625 noise w/cap. These are the only ones listed on this form.

11.) Click on **done**. On the 310 components menu press **Return.**
On the main diagnostics menu press **Quit.**

12.) Open the instrument door, the heat plate door, and the laser window door and place the regular capillary in front of the camera lens. Close all doors.

If any of the values fail call technical service.

Initials: RCJ **Date:** 5/7/99

QC162 DNA Sequencer (ABI 377): Maintenance

There are no diagnostic tests to be performed for the ABI 377 DNA Sequencer. Check, and if necessary clean all instruments, and sign the maintenance log. Two maintenance procedures are performed monthly and are described below. This information should be documented on a Maintenance Log sheet (F165) and filed in the ABI 377 Maintenance Log Binder.

Refilling the Water Reservoir- once a month and if the water level drops below one third. The ideal level for the water reservoir is between one third and two thirds full.

1. The water reservoir is located in a compartment on the right side of the instrument.

2. Make sure the pump is not running.

3. Open the compartment door. Unscrew the plastic bottle and remove it by pulling downward. Place a papertowel under the tubes connecting the reservoir to the pump.

4. Discard the old fluid, and rinse out the bottle. Fill the reservoir up to the mark (corresponds to 600 mL) with dH_2O, and add 50 mL of antifreeze.

5. Replace the reservoir, being sure to insert the two tubes before you screw it into place.

B **Review The QC Check Log**- once a month

1. Review the actual Prerun and Run values for all instruments, starting with the last QC check off. The values should be in the following range:

	Prerun	Run
E.Voltage (kV) -	3.00 ±0.05	3.00 ±0.05
Current (mA) -	10 - 15	30 - 50
Power (W) -	9 - 15	95 - 160
Laser Power (W) -	40.00 ±0.05	40.00 ±0.05

2. If any values are out of range, review the laboratory sheets, and the analysis results for the run in question. Determine possible sources for the out of range values, test and discard suspicious reagents lots.

3. Date and initial last entry that was checked.

Initials: RCS **Date:** 5/17/99

QC167 Gel Electrophoresis (ABI 377): Plate Preparation

Each new set of plates has to be treated with NaOH. This process does not have to be repeated.

A set of plates consists of one backplate and a notched front plate. The insides that will be in contact with the gel have to be treated. To mark which sides have to be the insides, the outside of the plates get etched in the following way:

Notched plate - an "L" for left on the left upper side, an "R" for right on the right upper side.
Plain plate - a mirror image "L" on the right side, and a mirror image "R" on the left side.
This way the "L"s and "R"s should be readable when the plates are placed correctly.

Place the plates on a sheet of bench paper with the side of the plates that is not etched facing upwards. **CAUTION:** Wear protective goggles, gloves and a lab coat before handling sodium hydroxide!!! Pour 10mL of 10N NaOH on the plate and distribute it evenly using a bundle of large Kimwipes. Rub the plate for approximately one minute in every direction. Rinse the plate off with plenty of tap water followed by a final rinse with deionized water. Repeat for the second plate.

Wash plates by hand throughout the entire procedure. Do not use the dishwasher.

The plates can be used immediately after treatment.

Initials: PCJ **Date:** 5/7/99

Calibration & Maintenance

Micropipettes are sent to an outside vendor twice a year for calibration.

Each station is equipped with a set amount of pipetman. During the time of calibration, complete sets of pipetman are replaced with a substitute set consisting of pre-calibrated pipetman that are reserved for this particular function. The rotation of the pipetman are carried out in the following order: starting from unamplified DNA stations and ending with amplified DNA stations. The pipetman from several stations can be removed and sent for calibration at one time.

Any micropipette transfer to or from service for any reason (i.e. repair, calibration, return from calibration) must be documented on the respective Micropipette Maintenance Log (F170). These sheets are located in the Micropipette Calibration QC Log binder. This binder is organized by workstation (e.g. pipetman at the chelex station, pipetman at the amplification station, etc.).

Micropipettes are prepared by wiping the outer shaft with 10% bleach and then followed with a final wipe using 95% ethanol.

Package micropipettes in bubble wrap packaging material before shipping out.

The substitute set is rotated to the next station once the pipetmen that were sent out for calibration are returned back to their respective station.

Gravimetric Check of Pipetman Accuracy

The table on the following page shows the performance specifications for the various pipetman that are being used in the laboratory. These specifications show levels of tolerance at various points on a given pipetman's range. If measured values differ significantly from the specifications, the pipetman in question will be removed from laboratory use and included in the next shipment of pipetman for calibration.

Table: Pipette Performance Specifications

Type	Volume Setting (µL)	Percent Error	Allowable Range (µL)
P-1000	1000	≤ ±2.0	980-1020
	500	≤ ±2.0	490-510
	200	≤ ±2.0	196-204
P-200	200	≤ ±2.0	196-204
	100	≤ ±2.0	98-102
	50	≤ ±2.0	49-51
P-100	100	≤ ±2.0	98-102
	50	≤ ±2.0	49-51
	20	≤ ±2.0	19.6-20.4
P-20	20	≤ ±2.0	19.6-20.4
	10	≤ ±2.0	9.8-10.2
	2	≤ ±10	1.8-2.2
E-10	10	≤ ±2.0	9.8-10.2
	5	≤ ±5.0	4.75-5.25
	2	≤ ±10	1.8-2.2
Repeater	10 (500µL tip)	≤ ±2.0	9.8-10.2
	30 (500µL tip)	≤ ±2.0	29.4-30.6
	50 (500µL tip)	≤ ±2.0	49-51
	50 (2.5mL tip)	≤ ±2.0	49-51
	250 (12.5mL tip)	≤ ±2.0	245-255

P - Rainin Pipetman
E - Eppendorf Ultra-micropipette
Repeater - Eppendorf Repeater Pipette

Initials: RCS **Date:** 5/7/15

QC230 P30 Plate Reader Diagnostic Tests

Microwell (microtiter) plate reader(s) should be tested monthly for **linearity**, **repeatability** of readings, and **calibration**.

Linearity is determined by the relationship of the calibrator absorbance (well No. 2) to the p-nitrophenol (PNP) concentrations in the remaining wells.

Repeatability is determined by comparing the absorbance of a given well in the strip when the strip is read twice in succession.

Calibration is determined by measuring the absorbance of the calibration well (well No. 2) and comparing it to the acceptable absorbance range assigned to the Microwell reader. The acceptable range is determined by the Microwell reader manufacturer.

NOTE: PNP IS TOXIC. IT IS HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. IRRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE MUTAGEN. USE APPROPRIATE PRECAUTION WHEN HANDLING AND WASH HANDS THOROUGHLY AFTER USE.

Test Materials/Supplies

AccuChrome™ 405 Microwells Kit
Deionized Water
Parafilm™
Linearity/Repeatability and Calibration Record Sheets (found in Microwell kit)

Procedure

1. Remove one Microwell strip from the kit. Gently tap the bottom of the strip on the counter to settle PNP in the wells (this is to prevent loss of powder on opening). DO NOT remove the tab on the Microwell strip.

2. Gently remove plastic and paper covering the strip. Keep the strip right side up.

3. Reconstitute each well with 200 µl of deionized water.Pipet carefully to avoid splashing, bubbles, or overfill. Use a calibrated micropipet. **DO NOT** touch the bottom of the microwell with the pipet tip. **DO NOT MIX.**

4. Place the wells strip in the microtiter plate designed for these well strips. The well containing the blank (next to the calibrator) should be in the A1 position in the plate. Gently cover all wells of the strip with Parafilm™ to prevent evaporation. Let stand on bench top for two hours at room temperature (18-26°C). **DO NOT** disturb during incubation. Turn the plate reader on 15 minutes before the two hours are up in order to give the machine sufficient time to warm up. After 2 hours, remove Parafilm™, avoiding splashing.

5. When the two hours are up, place the microtiter plate with the test wells into the plate reader (The A1 position should be in the upper left hand corner). Press the **FUNCTION** key. Press the **PRINT ANALYSIS** key. The flashing square next to Analysis Parameters will be flashing the number one. Press No. 2 so the square flashes the number two. Press **ENTER.** The flashing square will now flash on the Format number. The Format number should flash the number one. Press **ENTER.** The Reference number, once the Analysis Parameters has been set, should default to read the wells at 595nm. Check to make sure this is so. Press the **PRINT ANALYSIS** key to ensure all parameters are correct.

6. Press **START** to begin the absorbance reading of the microwells. Press **FUNCTION**, then the +/- key to print the results. Repeat the reading of the wells by pressing the **START** button again and then print the second set of results as well.

Calculations

1. **Linearity Data Record**

a. Calculate the average concentrations for replicate wells. Then calculate the average concentration of wells 3,4; of wells 5,6; of wells 7,8; and wells 9,10,11.

Example:

Average Concentration of well 3 = 25.4
Average Concentration of well 4 = 25.6
Average concentration of wells 3 & 4: (25.4 + 25.6) / 2 = 25.5

b. using the Linearity Graph Paper provided with the kit, plot the calculated average concentration on the vertical axis and the assigned concentration (see below) on the horizontal axis for each set of replicate wells.

Well No.	PNP Concentration (Units)
Well1:	0 (blank)
Well2:	50 (calibrator)
Well3:	25
Well4:	25
Well5:	50
Well6:	50
Well7:	100
Well8:	100
Well9:	200
Well10:	200
Well11:	200
Well12:	0 (blank)

c. All values must fall within the shaded area on the Linearity Graph Paper. This means the instrument has acceptable linearity (+/- 10%) variation.

Specifications

Loss of linearity is an indicator of stray light due to filter deterioration. If the values fall outside the shaded area on the Linearity Graph Paper, the test must be repeated. If the repeat test values are still outside the shaded area on the Linearity Graph Paper, the instrument must be serviced and not allowed to be used for casework until it has passed the test.

2. **Repeatability Data Record**

a. Calculate the difference between the absorbance readings for each of the strip.

Example:

Reading	Well No.	Absorption	Difference
1st	3	.243	
2nd	3	.243	0.000
1st	4	.244	
2nd	4	.245	0.001

b. Record the difference for each well in the appropriate space on the second page of the report (the Repeatability Record Sheet on the back of the Linearity Record Sheet).

Specifications

To ensure repeatability of readings, the difference in absorbance of each well between the two readings must be within the acceptable range as indicated on the Linearity Graph Paper (Repeatability section). If the difference is not within the acceptable range, there is a loss of repeatability of the readings.

If the repeatability is not within the accepted range, the test must be repeated. If the repeat test results are still out of the accepted range, the instrument must be serviced and not be used for casework.

3. **Calibration Data Record**

a. AccuChrome™ Microwell strips calibration assignments are lot specific. Use

calibration ranges assigned on the Calibration Sheet included in each kit.

b. Recorded absorbance of the calibrator (well No. 2) of the first strip in the column labeled Strip 1 if you are using the first strip in a new kit. If previous strips have alrea been used, record the average absorbance of well number two for this run in the appropriate strip # column on the Calibration Record Sheet.

c. When the first strip in a kit is used set upper and lower limits for absorbance by drawing a line 0.040 absorbance units above and below the observed absorbance for the calibrator (well No.2). Absobances of all remaining strips should fall within the drawn absorbance limits.

Specifications

If the absorbance of the calibrator (well No.2) falls within the range on the Calibration Record Sheet contained in the kit (as established by Sigma Diagnostics) there is no significant change in the calibration performance of the instrument. The acceptable range incorporated the expected variation due to the strips, the dye, and run-to-run variation.

If the calibrator does not fall within the range on the Calibration Record Sheet, the test must be repeated. If the repeat test value falls outside the range on the Calibration Record Sheet, the instrument must be serviced and is not to be used for casework.

Documentation

File the Linearity/Repeatability Record Sheet that was filled out for this QC run with the Calibration Sheet that accompanied the kit for this lot of microwells. All sheets should be filed together in the P30 Plate Reader Maintenance Binder.

Initials: RCS **Date:** 5/7/15

QC235 P30 ELISA Disinfection

Disinfection of the P30 plate washer should be done weekly to insure good working order of this instrument. Documentation for the performance of this procedure is recorded on the Plate Washer Maintenance Log Sheet (F180) and filed in the Plate Washer Maintenance Log Binder.

The protocol for this procedure is as follows:

1. Prepare a 10% solution of bleach (100 ml of bleach, 900 ml of dH_2O).

2. Under the SELECT function press the up arrow to reach the DISINFECTION program. Press YES.

3. The machine will prompt the connection of the disinfectant (the 10% bleach solution). Place the designated wash hose into the bottle of prepared bleach mixture (DO NOT pour the bleach mixture into the designated wash container that came with the machine or it will have to be thoroughly rinsed when disinfection is complete). Press YES.

4. The machine will indicate that the pump is priming. Disinfection will then occur for 30 minutes.

5. The machine will prompt the connection of the rinse. Place the wash hose into either the washer's designated rinse bottle filled with dH_2O or a plain bottle filled with dH_2O. Press YES.

6. The machine will indicate that the pump is priming. Prime the plate washer multiple times to ensure that the machine and the wash hose are free of the 10% bleach solution.

7. The SELECT function will return at the RUN program. You may now turn the plate washer off.

Initials: QCS **Date:** 5/7/99

QC245 pH Meter

A two-point calibration is done weekly using the pH meter and standard pH solutions. This information is documented on a pH Meter Calibration Log (F175) sheet and filed in the pH Log & Water System Binder.

Two-point Calibration

Choose standard buffer solutions for a two-point calibration which bracket the expected final pH of the solution to be measured. (i.e. use pH 7 and 10 standard buffers for a solution with final pH of 8.) Press STNDBY/MEAS button before the electrode is removed from any solution. Do not allow electrode to dry out.

Fill the electrode with saturated KCl solution if necessary.

Press STNDBY/MEAS button.

Press TWO POINT CAL button. The display asks for the pH of the first standard solution. Enter the pH value of the standard solution and press ENTER.

Press STNDBY/MEAS button.

Rinse the electrode with deionized water. Blot dry outside of electrode.

Place the electrode in fresh standard buffer solution and press STNDBY/MEAS button

The meter will stabilize the mV reading at that pH.

When the readout is stable and 3 asteriks are visible, press ENTER.

The display asks for the temperature of the reading. Enter the room temperature (a value of 24.0°C is adequate for these measurements).

The display asks for the pH of the second standard solution. Enter the pH value and press ENTER.

Press STNDBY/MEAS button.

Rinse the electrode with deionized water. Blot dry outside of electrode.

Place the electrode in the second standard buffer solution and press STNDBY/MEAS button.

The meter will stabilize the mV reading at that pH.

When the readout is stable and 3 asteriks are visible, press ENTER.

Enter the temperature.

Once the measurement has stabilized and 3 marks (<|) appear, rinse the electrode with deionized water. Blot dry outside of electrode.

The meter is calibrated before routine measurements.

Routine pH Measurements

Fill the electrode with saturated KCl solution if necessary. When fresh KCl is added, it is a good idea to mix the solution in the electrode by slowly inverting the electrode several times before continuing.

Calibrate the pH meter.

Rinse the electrode with deionized water. Blot dry outside of electrode.

Place the electrode in the solution. When the measurement has stabilized and 3 marks (<|) appear, record the measurement.

Calibration & Maintenance

The pH electrode must be kept filled with saturated KCl solution. This solution is approximately 30% KCl. The electrode is stored in a 2% KCl solution made from the saturated KCl filling solution (NOT deionized water or pH 7.00 standard solution). Do not leave electrode in deionized water for long periods of time.

When measuring the pH of large volumes, the pH electrode must be held in place. The electrode can be damaged if it is hung over the edge of the container and allowed to stir with the solution.

If the pH reading drifts or requires a long time to stabilize, the electrode bulb may need to be rejuvenated in 1 M HCl or the electrode may need to be replaced. Refer to the Beckman insert for further details of electrode maintenance.

Initials: RCJ **Date:** 5/7/89

QC020 SAVANT UVS400 Freeze Drier/Vacuum Pump

1. Turn on main power to allow unit to cool. Wait 30 minutes before use.
2. Place samples in centrifuge
3. Set drying rate at medium.
4. Turn rotor on.
5. Turn on vacuum switch.
6. Place arrow perpendicular to hose 90° clockwise. Check to make sure cover on rotor cannot open.
7. Allow samples to dry for appropriate time.
8. Turn off vacuum. Place arrow parallel with hose. (270° turn clockwise)
9. Shut off rotor and remove samples.
10. Turn off power.
11. Detach condensation bottle from unit and check for condensation. If condensation is present, dry bottle and reattach to unit.**

** **THIS STEP MAY BE DONE PERIODICALLY**

Initials: AC **Date:** 5/7/99

Refrigerators & -20°C Freezers

A digital thermometer is used to measure refrigerators and -20°C freezers. The refrigerator and -20°C freezer temperatures are recorded daily during the Monday through Friday work week.

Place the probe into the refrigerator or -20°C freezer and close the door. Make sure the door seal closes tightly around the probe wire. Allow the probe to equilibrate 5 - 10 minutes. The probe should not be removed from the unit.

Measure the temperature and document in the respective Refrigerator and Freezer (-20°C) Temperature Control Log (F190 and F115, respectively) sheet for that unit.

-80°C Freezers

An Omega thermocouple thermometer and an Omega thermocouple probe (type T-Brown) is used to measure -80°C freezers. The -80°C freezers are monitored daily during the Monday through Friday work week.

Place the probe into the -80°C freezer and close the door. Make sure the door seal closes tightly around the probe wire. Allow the probe to equilibrate 5 - 10 minutes. The probe should not be removed from the unit.

Measure the temperature and record reading in the monthly Freezer (-80°C) Temperature Control Log (F120) sheet for that unit.

Air Humidity & Temperature

A digital hygrometer/thermometer is used to measure the north, south, and southeast rooms of the laboratory. The room temperature and percent humidity is recorded daily during the Monday through Friday work week.

Place the probe on any surface and allow it to equilibrate for 5 - 10 minutes. Measure the temperature and percent humidity and log in the Temperature Control Log (F120) sheet for that room.

Water Baths & Heat Blocks

An Omega thermocouple thermometer and an Omega thermocouple probe (type T-blue) are used to measure the temperature of the water baths and heat blocks. Each probe is calibrated before use (see QC280). Temperature measurements are recorded each day the water bath is used. Temperatures are recorded daily during the Monday through Friday work week for the heat block.

To measure the temperature, turn the water bath or heat block on (if necessary) and allow it to equilibrate for at least 15 minutes. The probe is mounted in the water bath or positioned in the heat block.

When the temperature has stabilized, record the temperature reading on the appropriate Temperature Control Log sheet or Water Bath Temperature Control Log (F230). To measure the thermocouple temperature, plug the probe into the correct position in the meter (silver-colored constantan on the left, copper on the right). Record the reading. The thermocouple reading can be corrected using the slope and y-intercept values calculated from the probe calibration (see QC280).

Unit	Acceptable Thermocouple Reading
QuantiBlot Water Bath	50 ± 1°C
56°C Heat Block	56 ± 3°C
65°C Heat Block	65 ± 3°C
95°C Heat Block	95 ± 3°C
100°C Heat Block	100 ± 3°C

Calibration

All digital thermometers and hygrometer/thermometers are sent out for calibration against a NIST traceable standard to an outside vendor once a year. Documentation of calibration is recorded on an appropriate log sheet (F165) and filed in the Temperature Equipment Maintenance Log Binder.

Type T-Blue thermocouples which are used to monitor waterbath and heat block temperatures, are calibrated yearly against a NIST traceable mercury thermometer as described in QC280.

Type T-Brown thermocouples are used to measure temperatures of the -80°C low temperature freezers. Since an exact low temperature of these freezers is not critical (eg. for storage of forensic DNA extracts), Type T-Brown thermocouples are not calibrated. However, the performance of the Type T-Brown thermocouple is verified yearly as described in QC285.

If a suspicion arises of the performance of any of the digital thermometers, hygrometer/thermometers, Type T-Blue or T-Brown thermocouples during use, that particular temperature measuring device will be taken offline and recalibrated or reverified to insure that it meets proper specification.

Initials: RCJ **Date:** 5/7/99

QC280 Thermocouple Calibration (Type T-Blue) **Page 1 of 4**

The Type T-Blue thermocouple is calibrated once a year against a NIST traceable thermometer, graduated to 0.1 °C over the range -1.0 to 101.0°C. Before beginning the calibration procedure, the thermometer is checked by measuring two standard temperatures.

Thermocouple Temperature Response

Add 3 liters of distilled water to a 4 liter glass beaker.

Place the beaker on a stir plate.

Set up a clamp and ring stand behind the beaker.

Clamp the thermometer onto the ring stand and position it so that it can be submerged in the water.

With a twist tie, attach thermocouple near the bulb of the thermometer so that the thermocouple bead is close to but not touching the bulb.

Lower the thermometer, with attached thermocouple and wire, into the water. Tighten the clamp to hold the thermometer at the correct depth. The thermometer has an etched line 17 cm from the bulb which is the minimum level the thermometer must be immersed for accurate readings. Failure to immerse at the correct depth will result in incorrect results.

Plug the thermocouple into the socket of the thermocouple thermometer to be used during routine measurements.

Turn on the stir plate. Stir the water to the point where a shallow vortex forms. If necessary, adjust the stirrer during the procedure to keep the water well stirred. Thorough mixing will reduce temperature gradients near the thermometer.

Seven or eight comparisons of the thermometer and the thermocouple thermometer should be made, over a range of 25°C to 94°C. Temperatures must not be taken above 95°C because the formation of small vapor bubbles can cause fluctuations leading to variable temperatures.

The first measurement is made at room temperature. Record the reading from the thermometer and the thermocouple thermometer on the Thermocouple Calibration Log (F200). The probe measurements are recorded under the x-axis column, and the readings from the thermometer are recorded under the y-axis column.

Raise the temperature of the water approximately 10°C above room temperature by heating the stir plate.

When the temperature has risen several degrees, turn down the heat.

Check the immersion level of the thermometer. The position of the thermometer may have to be adjusted to compensate for evaporation of water.

If gas bubbles have formed on the thermometer or the thermocouple, gently tap the lower part of the thermocouple wire with a pencil to release them.

Check the temperature of the thermometer until successive readings show changes of less than 0.2°C in a 15 second period.

Once the temperature has stabilized, but at least one minute after any adjustment of the probe, record the readings of both thermometers.

Heat the water about 10°C more. Lower the heat until the temperature stabilizes, check the immersion level, remove any gas bubbles, and record the second set of readings.

Repeat this process until seven or eight temperature measurements have been recorded from 25°C to 95°C. For best results, the number of comparisons within a set should be a bit greater at the top of the range to compensate for a higher uncertainty of measurement. The multiple readings will partially overcome the uncertainty in reading the thermometer and provide confidence in the performance of the system over a range of temperatures.

Calibration Line

If the pairs of readings taken during the calibration procedure were plotted on a graph, thermocouple values along the x-axis and thermometer values along the y-axis, the points would fall along a straight line. This line is the calibration curve which relates observed temperature values measured by the thermocouple probe to standard temperatures. The calibration line is defined mathematically by the equation

$$y = mx + b$$

where m is the slope and b is the y-intercept.

The best fit line for the data can be calculated directly using the least squares method. The least squares calculation yields the slope and intercept necessary to convert thermocouple readings into standard temperatures as well as the correlation coefficient, r. The correlation coefficient gives a quantitative estimate of the goodness of fit. The closer the data points are to the best fit line, the higher the correlation coefficient. A perfect fit has a correlation coefficient of 1.

Calculations

The following are calculated and recorded on the Thermocouple Calibration Sheet (F010). The variable n is the number of data points collected during the calibration experiment, typically seven or eight.

The following are calculated the same way for the sets of x and y values. The discussion describes the calculations with respect to the x values only, assuming parallel calculations for the y values will be performed. Summation (x) is calculated by adding together the x-axis values. This is written in standard notation as

$$\text{sum}(x) = \Sigma\, x_i$$

Mean x equals summation (x) divided by n. This is written

$$\bar{x} = \frac{\text{sum}(x)}{n}$$

Summation (x^2) is the sum of the squares of the x values. All of the x values are squared first and then the squares are added together. This is written

$$\text{sum}(x^2) = \Sigma\, (x_i^2)$$

S_{xx} is defined as the sum of the squares of the x values minus the sum of the x values squared divided by n.

$$S_{xx} = \text{sum}(x^2) - \frac{[\text{sum}(x)]^2}{n}$$

Summation (XY) is calculated by multiplying the pairs of x and y values together and adding the products together.

$$\text{sum}(xy) = \Sigma\, x_i y_i$$

S_{xy} is defined as the sum of the x and y products minus the sum of the x values times the sum of the y values divided by n.

$$S_{xy} = \text{sum}(xy) - \frac{\text{sum}(x)\ \text{sum}(y)}{n}$$

The slope of the best fit line, m, is defined as

$$m = \frac{S_{xy}}{S_{xx}}$$

The intercept is calculated using the mean x and y values.

$$b = \bar{y} - m\bar{x}$$

Finally, the correlation coefficient is calculated using

$$r = \frac{S_{xy}}{(S_{xx} S_{yy})^{1/2}}$$

The slope is written with three significant figures. The intercept is rounded to the tenth's place. The correlation coefficient has a specification of >0.9999. If the calibration passes specification, the probe is ready for use.

Procedure for Type T-Blue Thermocouple

Poke a small hole through the center of the cap of a sterile reaction tube using a sterile needle.

Without bending the wire, pass the thermocouple through the hole from the top of the cap, so the soldered tip of the wire will be inside the tube when the cap is closed.

Tie an overhand knot in the insulated part of the wire. Carefully tighten the knot so that it fits inside the cap of the tube. The knot should not be so tight as to kink or break the wire. The knot prevents the wire from being pulled out of the tube during temperature measurements.

Check the length by closing the tube and pulling the knot against the inside of the cap. Enough of the thermocouple wire should remain below the knot so that the thermocouple is within 1 mm or so of the bottom of the tube; it may touch the tube wall slightly. Adjust if the length is too long or too short.

For the thermocycler probe, place 120 µL of deionized water into the tube and overlay with two drops of mineral oil. The mineral oil prevents evaporative cooling of the liquid inside the tube.

For the water bath probe, place approximately 1 mL of mineral oil into the tube.

Close the cap of the tube. The thermocouple tip should be just above or lightly touching the end of the tube. Do not seal the hole in the cap. If the cap is sealed around the thermocouple wires, the pressure in the tube at high temperatures will force liquid up between the sheath and the wire.

Initials: RC **Date:** 5/7/21

QC285 Thermocouple Verification (Type T-Brown)

Temperature probe operation is verified once a year.

Before beginning the verification procedure, the NIST traceable thermometer is checked by measuring two standard temperatures.

Mercury Thermometer Standardization

Place the NIST traceable thermometer in an ice water slurry. The etched line around the bottom of the thermometer must be at or below the level of the liquid. Allow the temperature to equilibrate. The thermometer must read between -0.2 and 0.2°C.

Place the thermometer in a boiling water bath. The etched line around the bottom of the thermometer must be at or below the level of the liquid. The thermometer must read between 99.8 and 100.2°C.

Record the results of the temperature check on the Thermocouple (Type T-Brown) Verification Log (F205).

Verification

Place the temperature probe in an ice water slurry along with a NIST traceable thermometer that has been previously standardized. Allow the temperature to equilibrate. The probe must read between -1 and 1°C.

If the probe is going to be used in the 0 to 100°C range, place the temperature probe in a boiling water bath. Allow the temperature to equilibrate. The probe must read between 99 and 101°C.

If the probe is going to be used in the -80 to 0°C range, place the temperature probe in a dry ice ethanol slurry. Allow the temperature to equilibrate. The probe must read between -78 and -74°C.

Record the results of the temperature check on the Thermocouple (Tye T-Brown) Verification Log (205). If the type T-brown probe fails verification, it is removed from service. The probe must meet the above specifications to be certified for use.

Initials: RG **Date:** 5/7/99

QC290 Thermocycler Block Cleaning

The wells of the sample block must be cleaned each month. Dirt, oil, and other contaminating agents collect in the sample wells, preventing the reaction tubes from seating properly. Maximum contact ensures optimum heat transfer from the block to the sample.

Documentation of Thermocycler Block Cleaning is kept in the Thermocycler Calibration and Maintenance Log Binder.

Procedure

NOTE: PROTECTIVE EYEWEAR MUST BE WORN WHEN CLEANING THE SAMPLE BLOCK. LIQUID MAY SPRAY OUT OF THE SAMPLE WELLS AS THEY ARE CLEANED WITH COTTON SWABS.

Prepare a 50% v/v isopropanol/water solution.

Clean excess oil out of the wells using kimwipes or cotton swabs.

Add one or two drops of the isopropanol solution to each well and carefully clean using cotton swabs. Rotating the swab helps to loosen material dried in the bottom. Wash the sides of each well with the isopropanol solution.

Remove excess liquid using a kimwipe or a dry cotton swab.

Check that there are no deposits left in the sample wells.

Clean the channels between the rows of the block using the same procedure.

If the deposits of dirt are heavy, it may be difficult to clean the wells. In this case, set the thermocycler to soak at 37°C. At a slightly warmer temperature, hardened deposits are easier to remove.

If the sample block has been contaminated with biological material, clean the wells using a 10% bleach solution, followed by a distilled water rinse. Dry the sample wells with dry cotton swabs or kimwipes.

Initials: RG **Date:** 5/1/98

There are six diagnostic tests run once a month. The test results are recorded on a Thermocycler Diagnostic Log sheet (F210).

To access the diagnostic test files, use the following commands.

Press **File, Yes**.

The following will appear on the display.

Select Function
CONFIG-DIAGNOSTIC

Press **No**.

This moves the cursor to the "Diagnostic" option.

Press **Enter**.

The following will appear on the display.

Diagnostic Tests
Enter test # (1-6)

Type the number of the test you want and press **Enter**.
To leave a test, press **Stop**.

Test 1: Display/Keypad Test

The machine first illuminates each block on the display board. The operator must watch to see that all the dots light up across the screen. Next, the operator checks each of the keys on the control board. As each key is pressed, the machine should display the corresponding command or number.

Test 3: Heater Test

This test measures the maximum heating rate. At the end of the test, the machine displays the time in seconds required for the first 15 degrees of temperature change, the temperature difference between the upper and lower temperature sensors just before the heaters go off (if applicable), and the heating rate. The heating time is a measure of the thermal time constant of the sensor/block assembly. If its value is not correct, a mechanical problem is indicated. The

temperature difference is an indication of proper sensor operation and installation. Before conducting the test, measure the line voltage with a voltmeter. Compare the results to the specifications.

Test 4: Chiller Test

This test measures the maximum cooling rate. The machine displays the sensor difference and cooling time similar to the heating test. Allow the machine to idle for at least 30 minutes before this test is run so that the coolant has time to reach operating temperature. Compare the results to the specifications.

Test 5 : Overshoot Test

This test measures the temperature overshoot on a set point step from 37 to 94°C. The block is set to 37°C for 1 minute then ramps up to 94°C. The overshoot past 94°C is shown on the display after 15 seconds. Compare the results to the specifications.

Test 6: Undershoot Test

This test measures the temperature undershoot on a set point step from 94 to 55°C. The block is set to 94°C for 1 minute and then ramps down to 55°C. The undershoot past 55°C is shown on the display after 15 seconds. Compare the results to the specifications.

Evaluation of Results

If all the results meet specifications, the thermocycler passes diagnostic testing. The Thermocycler (PE 480) Diagnostic Log (F210) is filed in the Thermocycler Calibration and Maintenance Log Binder.

If the results for any of the diagnostic tests fail to meet specifications, the thermocycler must be taken off-line for casework. Recent casework must be reviewed and selected samples may be retyped to confirm the results. Further testing may be necessary to rule out the possibility of human error. The test may not have been run properly or the results may not have been interpreted correctly. If after review the results fall consistently outside specification, the thermocycler must be tested before it can be put back on-line. If all the wells pass the test, casework may resume. If any of the wells fail the test, those wells must be taken out of service. The wells which pass the test can still be used event if there are wells on the same machine out of service.

Maintenance

Temperature verification and uniformity tests are done yearly according to the manufacture's instructions (Perkin Elmer, 1995b). These tests are performed using a digital thermometer and probe as part of a Temperature Verification System that was purchased from the manufacturer. The thermocycler must pass the specifications set by the manufacturer to be used online in forensic STR analysis.

Initials: RH **Date:** 5/17/05

QC300 Thermocycler Diagnostic Tests (PE 9600)

There are five diagnostic tests that are run for the GeneAmp PCR System 9600.

The heater, chiller, system performance and running the verify calibration diagnostic tests should be done monthly for the GeneAmp PCR System 9600 according to the GeneAmp PCR System 9600 Manual (Perkin Elmer, 1995a). The 9600 Thermocycler must pass all of these tests to be used for online forensic casework.

The test results are documented on a Thermocycler (PE 9600) Diagnostic Log (F215) and filed in the Thermocycler Calibration and Maintenance Log Binder.

In addition, temperature verification and uniformity tests are done yearly according to the manufacture's instructions (Perkin Elmer, 1994). These tests are performed using a digital thermometer and probe as part of a Temperature Verification System that was purchased from the manufacturer. The thermocycler must pass the specifications set by the manufacturer to be used online in forensic STR analysis.

Initials: RG **Date:** 5/7/15

QC310 Water Quality Maintenance

Changing Water Filters
Water filters should be changed once every two weeks. This is documented on a Maintenance Log (F165) and filed in the pH Log & Water Systems Binder. Use the procedure that follows to change filters:

1. Turn off the main water valve. Open deionized water valve and depress pressure release button (red button on dispenser) to relieve pressure in the housing.
2. Unscrew filter housing from cap, discard used cartridge and insert new cartridge (1 and 5 um).
3. Screw the housing onto the cap and hand tighten.
4. Open the main water valve slowly. Let the water run for 1-2 min. through the dispenser.
5. Turn off the deionized water dispenser.

Checking Water Quality
Water quality is checked weekly to include readings of total chlorine, free chlorine, total hardness, total alkalinity, pH and resistivity of the water using an Aquacheck strip and Myron L conductivity meter. Information is recorded on a Maintenance Log (F165) along with water filter information (if necessary) and filed together in the pH Log & Water Systems Binder.

Procedure
1. Take one strip from the bottle.
2. Turn on the distilled water
3. Pass the strip under water system
4. Remove (do not shake).
5. Hold strip level for 30 seconds.
6. Compare total hardness, total alkalinity and pH to the color chart shown on the bottle.
7. Record the readings on the log.
8. Again hold the strip under water system for 10 seconds.
9. Compare chlorine pads to the color chart.
10. Record readings on the log.

Checking Water Resistivity
1. Check batteries of the meter by pressing the button at the lower right corner of the meter. If the light is not visible change batteries.
2. Select range by turning the range knob at the lower left corner (x .1).
3. Rinse the cell cup three times with deionized water.
4. Then fill with deionized water to at least 1/4" above upper electrode.
5. Push button to read directly in microohms or megaohms.

Record the readings on the same Maintenance Log as for checking the Water Quality. File the Maintenance Log into the pH Log & Water System Binder.

Initials: **Date:**

Appendix C

This appendix shows a list of log usage and maintenance forms that are used in the OCME Forensic Biology Laboratory to provide records of equipment use, calibration, and maintenance. All of these forms can be accessed on the Forensic Biology computer network by following this path:

G: Users: Fbiology: Manual: Current: QC: C-forms: Fxxx

where xxx is the form number in question (eg., the name of the file name for the Balance Verification and Manintenance Log is F100).

Usage and Maintenance Log List

F100 Balance Verification and Maintenance Log
F105 Capillary Electrophoresis Diagnostic Log
F110 Capillary Electrophoresis (ABI 310) Usage Log
F115 Freezer (-20°C) Temperature Control Log
F120 Freezer (-80°C) Temperature Control Log
F125 Gel Electrophoresis (ABI 377) Parameters Log
F130 Gel Electrophoresis (ABI 377) Usage Log
F135 Heat Block (56°C) Temperature Control Log
F140 Heat Block (65°C) Temperature Control Log
F145 Heat Block (95°C) Temperature Control Log
F150 Heat Block (100°C) Temperature Control Log
F155 Hood Flow Rate Log
F157 Incubator Control Log (37°C)
F160 Kit Control Log
F165 Maintenance Log
F170 Micropipette Maintenance Log
F175 pH Meter Calibration Log
F180 Plate Washer Maintenance Log
F183 Raw Materials Log
F185 Reagent Inventory Log
F190 Refrigerator Temperature Control Log
F195 Temperature/Humidity Control Log
F200 Thermocouple (Type T-Blue) Calibration Log
F205 Thermocouple (Type T-Brown) Verification Log
F210 Thermocycler (PE 480) Diagnostic Log
F215 Thermocycler (PE 9600) Diagnostic Log
F220 Thermocycler File Log
F225 Thermocycler Usage Log
F230 Water Bath Temperature Control Log

Initials: RCJ **Date:** 5/17/99

Appendix D

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