

Forensic Biology Work Instructions

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\*Denotes section of the manual approved by the Biological Screening Technical Lead

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**Section 1 Chemicals and Reagents**

If the laboratory changes the preparation/verification procedures for a particular chemical/reagent, this change will be reflected in the chemical/reagent log and/or the verification paperwork.

Similarly, variations in vendor supplied materials (changes instituted by the vendor and outside of laboratory control) will be assessed to determine if the change adversely affects the laboratory analysis in which the reagent/chemical is used. This assessment will also be documented in the verification paperwork.

Such changes/modifications will be incorporated into this manual at the time of the next revision.

**Preparation and Verification of Reagents and Chemicals**

**AmpliAq Gold® DNA Polymerase**

(DNA critical reagent)

Purchased from Applied Biosystems and stored at -20°C; expires on date provided by manufacturer.

Verification

Amplify a positive control sample which may be the 9947A control DNA or an Internal Control Standard which is a sample whose genetic profile was previously established by the laboratory. The positive control can be a previously typed convicted offender sample / known casework sample / buccal swab from a laboratory employee). A negative amplification control (no template DNA is added to the reaction) using the new Taq Polymerase lot is also amplified.

Performance criteria for acceptance of the Taq Polymerase: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the positive control sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold.

The Taq Polymerase will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.

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**BCIP Solution (5-bromo-4-chloro-3-indolyl phosphate)**

Dissolve 0.025g BCIP in 50mL sodium acetate buffer (0.01M, pH – 5.5). Store at 2-8°C; solution expires 4 weeks from date of preparation.

Verification

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

**Buffer G2**

**(DNA critical reagent)**

(when purchased outside of a kit)

Purchased from Qiagen and stored at room temperature; expires on the date specified by vendor or 10 years from date received if expiration date is not specified by the vendor.

Verification

Extract and amplify a reference sample (a sample whose genetic profile was previously established by the laboratory e.g. a previously typed convicted offender sample / known casework sample / buccal swab from a laboratory employee). A corresponding reagent blank will also be processed with the new kit lot.

Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding reagent blank..

The reagent will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.

**Buffer MTL**

**(DNA critical reagent)**

Purchased from Qiagen and stored at room temperature; expires on the date specified by vendor or 10 years from date received if expiration date is not specified by the vendor.

Verification

Extract and amplify a reference sample (a sample whose genetic profile was previously established by the laboratory e.g. a previously typed convicted offender sample / known casework sample / buccal swab from a laboratory employee). A corresponding reagent blank will also be processed with the new kit lot.

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Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding reagent blank.. The reagent will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.

**Citric Acid Buffer (0.14M, pH 4.9)**

(for STMP solution #1 preparation)

Add 1.35g Citric Acid, anhydrous and 0.5g sodium hydroxide to 50mL de-ionized water. Adjust to pH 4.9 with NaOH /1:1 HCL.

**DTT (1M)**

(DNA critical reagent)

Working Solution

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

Verification

Extract and amplify a reference semen sample (a sample whose genetic profile was previously established by the laboratory). A corresponding reagent blank with the new DTT lot will also be processed with the reference semen sample.

Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding reagent blank.. The reagent will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.

**Ethanol (Absolute, 200 proof)**

(DNA critical reagent)

Verification

Extract and amplify a reference sample and a corresponding reagent blank with the new Ethanol lot.

Please see procedure and performance criteria for **Sterile De-ionized Water (H2O)**

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**EZ1 DNA Investigator Kit**

**(DNA critical reagent)**

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

Purchased from Qiagen and stored at room temperature. Reagent cartridges may be stored at 2-8°C for long-term storage. All components, except carrier RNA, expire on date provided by manufacturer.

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

Verification

Extract and amplify a reference sample (a sample whose genetic profile was previously established by the laboratory e.g. a previously typed convicted offender sample / known casework sample / buccal swab from a laboratory employee). A corresponding reagent blank will also be processed with the new kit lot.

Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding reagent blank..

The reagent will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.

**Hi-Di Formamide**

Purchased from Applied Biosystems. Aliquot (0.4mL and 1.0mL recommended) and store at -20°C. Aliquots are intended for one-time use and should not be re-frozen. Expires 10 years from date received.

**3% Hydrogen Peroxide**

Purchased locally and stored according to manufacturer's instructions. Does not expire as long as the expected results are given with positive and negative control samples (on each day the reagent is used, and prior to use on evidentiary samples).

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**1X Genetic Analyzer Buffer (GAB)**

In a sterile conical tube, add 5mL 10X Genetic Analyzer Buffer to 45mL sterile de-ionized water. Mix by inversion. This solution is stored at room temperature and should be prepared fresh and as needed (approximately weekly).

**Nuclear Fast Red stain**

Dissolve 5.0g of aluminum sulfate in 100ml of hot deionized water. Add 0.1g of Nuclear Fast Red. Stir and let cool. Filter the solution through filter paper and store at 4°C. Store at 2-8°C; expires one year from date of preparation.

**One-step PSA ABACards**

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions; expires on date provided by manufacturer.

Verification

A known human PSA standard and sample blank are to be run to verify a new lot(s) of cards. Dilute 2 – 10µl PSA standard L-F500 in 200µl of sterile deionized water to serve as a positive control. 200µl of sterile deionized water is placed into a tube to serve as a negative control. Follow the test procedure described in section 2 of this document. Record the lot number(s) and expiration date(s) and test results.

The control samples must give the expected results for test results on questioned samples to be valid.

**One-step HemaTrace ABACards**

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions; expires on date provided by manufacturer.

Verification

A known human blood standard (positive control) and a negative control (extraction buffer or deionized water) are run to verify a new lot(s) of cards. Follow the test procedure described in section 2 of this document. Record the lot number(s) and expiration date(s) and test results.

The control samples must give the expected results for test results on questioned samples to be valid.

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**Permout**

Purchased from a commercial vendor and stored at room temperature.

Working Solution: Permout diluted with Xylene if necessary.

Use until the reagents no longer adequately satisfy the purpose they are used for.

No expiration date although manufacturer provides one (refer to the DNA QA manual).

**Phenolphthalein (for Kastle-Meyer Test)**

Stock Solution

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

Working Solution

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

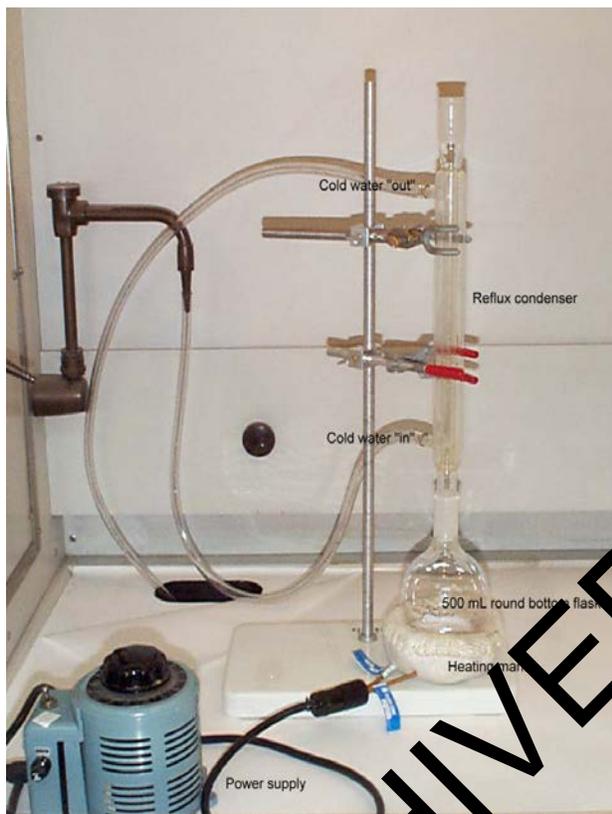
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**Figure 1. Phenolphthalein Stock Solution Preparation.**



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

### Picro-indigo-carmin stain

Add 0.33g of Indigo Carmine dye to 100mL of saturated picric acid. Filter the solution through filter paper. Store at 2-8°C; expires one year from date of preparation.

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

Components: 9947A DNA, 10X Primer Pair Mix, Gold ST\*R 10X Buffer, Allelic Ladder, Internal Lane Standard (ILS 600)

Purchased from Promega Corporation. 9947A is diluted, aliquotted and stored at -20°C. Other components are stored according to manufacturer's instructions. 9947A expires one year from date of dilution. Other components expire on kit expiration date provided by manufacturer.

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Use 975µL sterile de-ionized H2O to bring the 9947A to an appropriate concentration for setting up amplification reactions.

Amplify the 9947A positive control or an Internal Control Standard, a negative water control and a known sample twice using (1) the primers and buffer from the kit currently in use and the (2) the primers and buffer from the kit being verified, to compare amplification sensitivity and overall efficiency of amplification.

Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the positive control and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding negative water control blank.

The kit will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.

**Proteinase K Solution**

**(DNA critical reagent)**

(when purchased outside of a kit)

Purchased from Qiagen or another suitable vendor and stored at room temperature; expires on date provided by manufacturer or 10 years from date received if no expiration date is given.

Verification

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

Please refer to acceptance criteria for Taq Polymerase.

**Quantifiler (QE) Kit**

**(DNA critical reagent)**

Components: DNA Standard A, Human Primer Mix and Reaction Mix

Purchased from Applied Biosystems. DNA Standard A and Primer Mix are stored at -20°C. Reaction Mix is stored at 2-8°C. Components expire on date provided by manufacturer. Standard curve prepared fresh approximately weekly.

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Test new QF lot with a standard curve and NTCs. Expected results per manufacturer's information and specifications and /or comparison to previous lot of QF. Retest if the above criteria are not met with first testing and reject kit if the test lot fails after three verifications (prepare fresh standard curve after failure to check if problem was with the manner in which the reagent was prepared).

**Sodium Acetate buffer (0.01M, pH 5.5)**

(for BCIP preparation)

Dissolve 0.34g Sodium Acetate (anhydrous) in 200mL deionized water. Adjust the pH to 5.5. Bring to a volume of 250mL with deionized water. Store solution at room temperature; expires one year from date of preparation.

**Sodium Thymolphthalein Monophosphate**

Solution #1

Dissolve 0.01g Sodium Thymolphthalein Monophosphate in 50mL Citric Acid Buffer.

Solution #2

Add 0.53g Sodium Carbonate ( $\text{Na}_2\text{CO}_3$ ) and 0.20g Sodium Hydroxide (NaOH) to 50mL deionized water.

Store at 4° C; solutions are stable for 4 months.

Verification

Test the reagent with a positive semen control and a negative dH<sub>2</sub>O control prior to first use, and on each day used in casework

**Sterile De-ionized Water (H<sub>2</sub>O)**

**(DNA critical reagent)**

Fill glass bottles with nanopure de-ionized H<sub>2</sub>O. Autoclave for 30 minutes and store at room temperature. Expires 1 year from date prepared.

When autoclaving, include a Sterikon™ plus Bio-indicator, or equivalent. The DNA Technical Manager must approve use of reagents autoclaved without a Sterikon™ (or equivalent). This approval will be documented in the Reagent Log.

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After autoclaving, place the autoclaved ampoule, and an ampoule that was not autoclaved, in the 56°C incubator for 48 hours. Refer to the manufacturer's instructions to evaluate the results of the ampoules.

Verification

Amplify the 9947A control or similar acceptable positive control sample along with a negative amplification control using the new water lot.

Performance criteria for acceptanc : A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the positive control sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold.

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**Section 2 Screening of Biological Evidence**

**2.1 Alternate Light Source Examination using the Omniprint™ 1000A/1000B**

**General Safety Guidelines**

- It is essential that proper eye protection be provided for and worn by anyone operating an intense light source such as the Omniprint™ 1000B. Permanent eye damage can occur from specular (direct illumination to the eye) or reflected or refractive light hitting the eye. Label goggles for the specific wavelength. Do not allow use of inappropriate or incorrect goggles. Some goggles offer only a narrow band of protection.
- Remove all unnecessary reflective surfaces from the area of exam room. Avoid looking at reflections in shiny spherical objects such as door knobs, watch crystals, tools, jewelry, window panes, mirrors or any other surface that may reflect light.
- Exposing the skin to the beam of light (directly from the unit) can cause burns and other skin damage. There is no hazard with skin exposures to the beam emitting from the liquid light guide or fiber optic cables as temperatures are decreased, but the direct emission of the light from the discrete setting is very warm.

**Proper Operation of the System**

- Check to see that both switches are in the “off” position.
- Plug the unit into a three prong grounded outlet. If an extension cord is used, it must be a heavy duty grounded cord.
- Turn on the power rocker switch (marked “Line”). The switch will light, and the fan will begin to operate. You may now turn the lamp switch on (marked “Lamp”). The lamp should light within a few seconds.
- You may hear a ticking noise and see brief flashes of light while the lamp is attempting to ignite. This is normal.
- If the lamp fails to ignite within two minutes, and you hear a ticking noise, turn the unit off. You may have a bad lamp. Lamps are guaranteed for 500 hours of use, provided it has been used in the prescribed manner (periods of at least 15 minutes). Replace the lamp (see Lamp Changing Instructions provided with the manual). The lamp should be left running for periods of at least 15 minutes. The lamp must cool after it has been turned off and should not be restarted until it has fully cooled.
- Although the minimum suggested operating time is 15 minutes, it is important to note it is better to operate the lamp for continuous periods, rather than turning the lamp on and off. This procedure will increase lamp life.

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- If you do not hear a ticking noise and the lamp does not light, this is an indication that the power supply is not functioning properly. Please contact Omnichrome for further instructions.
- Operation in high ambient temperatures – the Omniprint™ 1000A/1000B is equipped with over temperature protection. If the instrument is being operated at ambient temperatures exceeding 100° F, the over temperature protector may shut off the lamp. When the unit cools sufficiently, the lamp will come back on automatically.

### Filter Selections

The wavelengths are selected by simply turning the knob marked “Wavelength Selector” in either direction. A green LED light will appear next to the selected wavelength.

- **450nm** band when used with yellow goggles or filters provides near UV excitation. Generally the optimum setting for the detection of physiological stains such as semen, saliva, urine and blood. Forensic odontologists and pathologists prefer this setting for bite mark and bruising detection and photographic documentation.
- Prior to the examination of casework evidence, a known semen stain control is examined with the wavelength set at 450nm. This ensures that the system is functioning properly. The stain must exhibit the expected fluorescence for the unit to be used in casework. This check is performed each day that the unit is in use and is documented in the bench notes.

### Shutdown Procedure

- Push the “Lamp” rocker switch off. You must now wait for the unit to cool down. The system includes dual fans for cooling.
- After feeling that the body of the unit and the exhaust are cool, the “Power” rocker switch may be turned off. The cool-down period is approximately 5 minutes.
- Never turn the lamp back on until the unit has completely cooled.
- If the unit is to be moved, remove the light guide(s) by gently pulling the cable out of the aperture. Gently wind the cable into a loose coil and place cable in a safe place or back into the case.
- The liquid light guide must not be wrapped or coiled too tightly as this can permanently damage the cable. The liquid light guide is very fragile and will become damaged if it is kinked, stepped on, bent, or frozen. Damage may not be evident, but there will be a decrease in output power (brightness).

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**2.2 Presumptive and Confirmatory Testing for Semen/Seminal Fluid**

**2.2.1 Detection of Acid Phosphatase using BCIP**

This is a presumptive test for the detection of seminal fluid.

**Reference**

F.S. Baechtel, J. Brown and L.D. Terrell, "Presumptive Screening of Suspected Semen Stain In Situ Using Cotton Swabs and Bromochloroindolyl Phosphate to Detect Prostatic Acid Phosphatase Activity," J. For. Sci., 32, pp. 880-887, (1987).

**Procedure**

- Place 200µL of BCIP substrate solution in labeled glass test tubes; one test tube for each sample. (Prepare a test tube for a positive control, a negative control, and for each Q sample).
- Negative Control: Moisten a sterile swab with a minimal amount of sterile deionized water. Place the swab in labeled test tube with BCIP solution. (A Negative Control is required for each run and should be the first sample prepared).
- Q Samples: Moisten sterile swab with a minimal amount of sterile deionized water. Lightly swab the questioned stain with the swab. Place the swab in a labeled test tube with BCIP solution.
- Positive Control: Moisten a sterile swab with a minimal amount of sterile deionized water. Swab the human semen sample with the swab for a positive control. Place the swab in labeled test tube with BCIP solution. (A Positive Control is required for each run and should be the last sample prepared).
- Put the test tubes in a rack. Place the rack in a 37°C water bath and incubate for 15 minutes. Document the water bath temperature in the log book. Record the following in case notes: lot # and expiration dates for the BCIP solution, lot # for the sterile water, and the sample # of the human semen stain used.

**Interpretation of Results**

A positive test result is an aqua (blue-green) color. The appearance of an aqua color indicates the presence of acid phosphatase activity. The positive control should give an aqua color and the negative control should not exhibit a color change. The controls must function as expected for the test results on Q samples to be valid.

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**Additional Notes**

- The BCIP procedure is not specific for semen.
- The BCIP procedure is 99% accurate in predicting a true negative stain, i.e. one out of 100 negative BCIP tests may be false. (reference Baechtel paper)
- The BCIP procedure has not been found to be useful in screening latex condoms. The laboratory has encountered condoms containing numerous spermatozoa that yielded negative BCIP results.
- This test should not be performed on body cavity swabs since spermatozoa may be found on vaginal swabs which do not test positive to the BCIP reagent.

**2.2.2 Detection of Acid Phosphatase using STMP**

This is a presumptive test for the detection of seminal fluid.

**References**

Roy, A. V., Brower, M. E. and Hyden J. E., "Sodium Thymolphthalein Monophosphate: A new Acid Phosphatase Substrate with Greater Specificity for the Prostatic Enzyme in Serum", Clinical Chemistry Vol. 17, No. 11, 1971.

Seiden, H. and Duncan, G., "Presumptive Screening Test for Seminal Acid Phosphatase Using Sodium Thymolphthalein Monophosphate", Proceedings from a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, FBI Academy 1983.

**Procedure**

- Positive & Negative Controls: A human semen standard control and sterile water blank must be tested each day that the test reagents are used in casework.
- Moisten a sterile swab with a minimal amount of sterile deionized water. Lightly stroke the questioned stain with moistened swab.
- Add one drop of Solution #1. Wait one minute.
- Add 2-3 drops of Solution #2.

Note: this method may also be performed by pressing a piece of filter paper moistened with deionized water over a suspected semen stain for a few seconds.

**Interpretation of Results**

The rapid development (within 15-30 seconds) of a blue color indicates the presence of a high concentration of acid phosphatase. Lower levels of acid phosphatase result in a green color. The positive control should exhibit rapid development of a blue color. The negative control should not exhibit a color change. The controls must function as expected for the test results on questioned stains to be valid.

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### Additional Notes

Acid phosphatase is present at high concentrations in seminal fluids. However, the enzyme is present in other body fluids (including vaginal fluid and feces) and in plants, fungi and bacteria. Sources of acid phosphatase other than semen generally produce slow, weak reactions.

### 2.2.3 Extraction of Suspected Semen Stains

After determining the possible presence of acid phosphatase in a stain, the following procedure will provide an extract of the soluble substances and a pellet of the particulate material for analysis. This procedure is also used for extraction of swabs.

#### Procedure

- Label a sterile 2.0 mL tubes for each sample to be extracted.
- Place a small cutting of the stain/swab(s) into each tube. Stains/swabs collected from the same area may be grouped.
- Add 200 $\mu$ L of sterile, deionized water to each cutting/sample in the tube and soak for at least 30 minutes at 37°C. The extraction may also be accomplished overnight at 37°C or in the refrigerator at 4°C.
- Label a microscope slide for each sample.
- Twirl the material in each tube with a clean toothpick or a sterile pipette tip and then spot 3 $\mu$ L of the extract onto a labeled microscope slide.

**Optional:** To improve recovery of the spermatozoa, the following procedure may be substituted:

- Agitate the cutting/sample using a clean toothpick for approximately one minute.
- Use a clean toothpick to transfer the cutting into a spin basket and insert into the 2.0mL tube containing the extract.
- Centrifuge for 1 minute (at speed sufficient to pellet the cellular material) and then discard the basket with the cutting.
- Resuspend the pellet and then spot 3 $\mu$ L of the extract onto a labeled microscope slide. The 3 $\mu$ L may be withdrawn from the pellet, but this should be clearly stated in the bench notes.
- The extract is retained in the refrigerator, until completion of the case, for possible P30/PSA testing.
- Follow the work instructions in the next section to stain and examine the slides for spermatozoa.

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If no spermatozoa are observed, a P30 analysis on the aqueous portion of the extract (supernatant) may be performed. Work instructions are provided in a later section of this document.

Microscope slides are packaged with the item after examination. Screening extracts are discarded after analyses are completed.

### 2.2.4 Staining Protocol

This is a differential staining process to aide in the conclusive identification of spermatozoa.

#### References

Stone, I.C., "Staining of Spermatozoa with Kernechtrot and Picroindigocarmine for Microscopical Identification", SWIFS, Criminal Investigation Laboratory, Sept. 1972.

Gaensslen, R., Sourcebook in Forensic Serology, Immunology, and Biochemistry, U.S. Government Printing Office, 1983.

#### Procedure

- Heat fix cells to a microscope slide by gently flaming or by placing the slide in a 37°C oven for 15 minutes.
- Slides may be examined microscopically prior to staining. Intact spermatozoa can often be detected. If intact spermatozoa are observed, staining is not required.
- Cover the slide/debris with Nuclear Fast Red stain and let stand for 10-15 minutes.
- Gently wash away the Nuclear Fast Red with deionized water.
- Immediately cover the slide/debris with Picroindigocarmine stain (PICS) and let stand for 15-30 seconds.
- Gently wash the slide with reagent grade ethanol. Allow to air dry.
- Add Permount and a cover slip.
- Examine the slide microscopically (200-400x) and record the results based on the interpretations given below.

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### Interpretation of Results

Nuclear material is stained red by the Nuclear Fast Red dye. Sperm heads are usually well differentiated with the acrosome staining significantly less densely than the distal region of the head. Picroindigocarmine stains the epithelial membranes green. Nuclei inside the epithelial cells may appear purple. Yeast cells also stain red; however, the stain is uniform throughout the cells and extends into polyp-like structures, which are occasionally observed with yeast cells.

If spermatozoa are detected, note if they are intact and score the number observed according to the following scale:

- <10 Few
- 1+ Hard to find
- 2+ Some in fields, easy to find
- 3+ Many or some in most fields
- 4+ Many in every field

Record the presence of nucleated epithelial cells (NECs) and non-nucleated cellular debris. NECs may also be graded using the scale given above.

### 2.2.5 Detection of PSA by the Abacus ABACard®

This is a rapid and sensitive detection method for PSA (P-30 or Prostate-specific antigen), a component of seminal fluid.

This test is normally performed after obtaining a positive presumptive test for seminal fluid (acid phosphatase) with negative sperm search results.

### References

Manfred N. Kochmeister, et al, "Evaluation of Prostate-specific Antigen (PSA) Membrane Test Assays for the Forensic Identification of Seminal Fluid", J Forensic Sci 1999;44: 1057-1060.

J. Kearsey, et al, "Validation Study of the 'Onestep ABACard® PSA Test' Kit for RCMP Casework", Can. Soc. Forens. Sci. J. Vol. 34. No 2 (2001) pp. 63-72.

Theresa F. Spear and Neda Khoskebari, "The Evaluation of ABACard® p30 Test for the Identification of Semen", The CACNews 1<sup>st</sup> Quarter 2001, pp. 22-24.

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### Procedure

- This procedure is done following extraction and a microscopic examination as previously described.
- Retrieve the extract prepared in section 2.2.3.
- If not already done, place the substrate into a spin basket and centrifuge the sample for 3-5 minutes.
- Remove the device and dropper from the sealed pouch. Label the PSA card with the case number, item number, your initials and the date.
- If refrigerated, allow the sample(s) to come to room temperature prior to testing.
- Add 4-5 drops of the extract, using the dropper provided, into the sample region (S) on the card.
- Allow the sample to stand for 10 minutes. Positive results can be seen as early as 1 minute depending on the P-30 concentration.
- The results of the test are documented using digital imaging and included in the analyst's bench notes.

### Interpretation of Results

Positive: The formation of two pink lines, one in the test area (T) and in the control area I is a positive result, indicating the concentration of P-30 is at least 4ng/ml. Another analyst should confirm weak results. This verification will be documented in the bench notes.

Negative: The formation of only one pink line in the control area I indicates a negative test result. This may indicate that (a) No PSA is present above 4ng/ml or (b) presence of "high dose hook effect". Presence of "high dose hook effect" may give a false negative result due to the presence of high concentrations of PSA in the sample. If this is suspected, the sample should be retested using a 1:10 to a 1:1,000 fold dilution of the sample in question using the remaining 100µl of sample.

Inconclusive: There is no formation of a pink line in either the test area (T) or the control area I of the card. Repeat the test and reexamine the test procedure carefully.

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### 2.3 Presumptive and Confirmatory Testing for Blood

#### 2.3.1 Detection of Heme with Phenolphthalein (Kastle-Meyer Test)

This is an oxidative test for the presumptive identification of blood based on the catalytic activity of the heme group of hemoglobin. If the sample is of a limited nature and the presumptive test is likely to consume sufficient sample to prevent successful DNA typing, then the presumptive test should not be performed. The limited nature of the sample will be documented in the analyst's bench notes.

#### References

Culliford, B., The Examination and Typing of Bloodstains in the Crime Laboratory. U.S. Government Printing Office, 1971.

Saferstein, R., Forensic Science Handbook Volume I, Prentice Hall, Inc., 1983.

Gaensslen, R., Sourcebook in Forensic Serology, Immunology, and Biochemistry. U.S. Government Printing Office, 1983.

#### Procedure

- Record all lot numbers and expiration dates.
- Positive & Negative Controls: A human blood standard control is swabbed and tested each day that the presumptive test reagents are used. A swab moistened with the same lot of sterile water used for the questioned samples is also tested to function as a reagent control.
- Questioned samples: Moisten a sterile cotton-tipped swab with sterile water. Rub the swab over the suspected bloodstain. Stains may also be sampled with a dry swab or a folded piece of filter paper, or by teasing a few stained fibers from the substrate.
- Add 1-2 drops of the phenolphthalein working solution to the swab. Wait a few seconds and observe any color changes.
- Add 1-2 drops of the 3% hydrogen peroxide solution.

#### Interpretation of Results

The appearance of a rapidly developing pink color after the addition of 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a presumptive positive test for the presence of blood. A pink color forming after one minute should not be considered as a positive result, as auto-oxidation can occur in air and light.

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The positive control must yield a positive result (i.e. rapid pink color change) and the negative control give no color change, for the test results on evidentiary samples to be valid.

Color catalytic tests are very sensitive, but not specific. The positive color test alone should not be interpreted as positive proof of blood. The major sources of “false positive” reactions are chemical oxidants and vegetable peroxidases. Color development before the addition of H<sub>2</sub>O<sub>2</sub> may be due to the presence of chemical oxidant. Fruit and vegetable peroxidases react similar to blood but slower and more weakly.

Additionally, the presumptive test for blood is not species specific. Blood and other tissues from animals besides humans will also give a positive reaction with this test.

A negative result is indicative of the absence of detectable quantities of heme or its derivatives.

### 2.3.2 Detection of Human Hemoglobin (hHb) using OneStep ABACard HemaTrace

This test is a simple, rapid and a sensitive confirmatory test for human (primate) blood.

#### References

Manfred N. Hochmeister, et al. “Validation Studies of an Immunochromatographic 1-Step Test for the Forensic Identification of Human Blood,” J Forensic Sci 1999;44:1057-1060.

#### Procedure

- Place a portion of the suspected bloodstain or swab into an entire labeled tube of the extraction buffer provided.
- Allow the sample(s) to extract for 1 – 5 minutes.
- If refrigerated, allow the sample(s) to warm to room temperature.
- Remove the device/card and dropper from the sealed pouch and label the HemaTrace card with the lab number, item number, your initials and the date.
- Add 4-5 drops of the extract, using the dropper provided, into the sample region (S) on the card.
- Allow the sample to stand for 10 minutes. Positive results can be seen as early as 2 minutes depending on the hHb concentration.
- Document the test results by digital imaging and include in the bench notes.

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**Interpretation of Results**

The control line in the control area I is an internal procedural control. A distinct pinkish line will always appear if the test has been performed correctly and the reagents are working properly. If the control line does not appear, the test is invalid and a new test must be performed.

Positive: The formation of two pink lines, one in the test area (T) and in the control area I is a positive result, indicating that the human hemoglobin concentration is at least 0.05µg/ml. Another analyst should confirm weak results. This verification will be documented in the bench notes.

Negative: The formation of only one pink line in the control area I indicates a negative test result. This may indicate that:

- No human hemoglobin is present above 0.05µg/ml.
- Presence of “high dose hook effect”. Presence of “high dose hook effect” may give a false negative result due to the presence of high concentrations of human hemoglobin in the sample. If suspected, the sample should be retested using a 1:100 or 1:1,000 fold dilution of the sample.

Inconclusive: There is no formation of a pink line in either the test area (T) or the control area I of the card. Repeat the test and re-examine the test procedure carefully.

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**Section 3 DNA Extraction**

**3.1 Qiagen BioRobot EZ1 Advanced-XL DNA Extraction**

**3.1.1 General Instructions:**

- Use the Trace or Large Volume protocols for all questioned and known forensic casework samples.
- The Large Volume protocol requires an additional reagent blank because of the addition of Buffer MTL after digestion. When using the Large Volume protocol, be sure to aliquot enough dilute G2 to your reagent blank tube ( $\geq 200\mu\text{l}$  of dilute G2 buffer) before digestion to have enough post-digestion solution to extract two separate reagent blanks to accompany the Trace and Large Volume protocols, respectively. Alternatively, a second reagent blank tube can be set up prior to digestion and used exclusively as the reagent blank for Large Volume protocol sample(s).
- Questioned samples shall be eluted in TE buffer.
- Known samples may be eluted in water or TE buffer.
- Elution volumes are selected based on sample type and/or quantity:
  - Larger elution volumes ( $200\mu\text{l}$ ) may be selected for large blood stains, 4+ sperm samples, 4+ epithelial fractions and reference samples
  - Smaller elution volumes ( $50\mu\text{l}$ ) are recommended for contact DNA samples and samples with few sperm or epithelial cells.
  - Larger elution volumes ( $200\mu\text{l}$ ) are recommended for known reference samples.
  - If the entire sample was consumed, the total elution volume shall be no less than  $100\mu\text{l}$
  - If more than one elution volume is used in a set of extractions, the corresponding reagent blank(s) should use the most stringent elution volume used in that set.

**3.1.2 Casework Samples – Direct (non-differential) Extraction**

1. Prepare the pre-digest solution:
  - (Number of samples + 3) x  $230\mu\text{l}$  G2 buffer
  - (Number of samples + 3) x  $230\mu\text{l}$  sterile de-ionized water
  - (Number of samples + 3) x  $10\mu\text{l}$  Proteinase K
2. Add enough pre-digest solution (no less than  $450\mu\text{l}$ ) to each sample cutting to allow for at least  $200\mu\text{l}$  of free liquid in the tube after absorption by the substrate.

**Note:** for hair samples and nail clippings\*, also add  $10\mu\text{l}$  DTT.

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(\* when the source or owner of the nail needs to be determined)

3. Incubate at 56°C. Known samples shall be incubated for at least one hour. Question samples will typically be incubated overnight. Exceptions will be noted in the bench notes.
4. Following incubation, transfer 200µl of the digest buffer into a sterile 1.5mL screw-cap tube. If absorption by the swab is unusually great, transfer as much of the digest as possible, up to 200µl. For all casework questioned samples, add 1 µl carrier RNA solution to the transferred digest solution. It is not necessary to add carrier RNA to reference sample extracts.

**Note:** If necessary, prepare the carrier RNA solution by reconstituting a previously verified lot of lyophilized carrier RNA in 310µl of sterile, deionized water. Vortex, and spin briefly. Prepare 20µl, single use aliquots in 0.5mL tubes and store at -20°C.

**Option 1 – Large-Volume Protocol:**

- For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500µl of the digest solution to an EZ1 sample tube, add 1µl carrier RNA solution, and add 400µl of Buffer MTL.
- Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in 50µl TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.

**Option 2 – Large-Volume Protocol for Consumed Samples:**

- If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes – with no more than 500µl in each tube), add 1µl of carrier RNA solution and add 400µl of Buffer MTL.
- Run the Large-Volume protocol and elute in 100µl TE buffer.
- On occasion, use of more than one tube may be required to digest and extract the entire sample efficiently. In such situations, the EZ1 extracts obtained from all such digests may be combined in one tube and proceed to the quantitation step.  
**NOTE:** If any sample is extracted using the Large-Volume protocol and eluted in 50µl, then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted 100µl. If no other Large-Volume protocol samples are extracted,

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*ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in at least 50 $\mu$ l.*

5. Reagent cartridges may be stored at room temperature (short term) or between 2°C and 8°C (long term). If the reagent cartridges have been stored between 2°C and 8°C, warm them up by leaving them at room temperature for several hours or placing them in a 37°C incubator for approximately 1 hour.
6. Turn on the power switch on the back of the instrument.
7. Directions are displayed on the screen on the front of the instrument. Press the START button to select a protocol (Trace or Large-Volume), elution buffer and elution volume.

**Note:** If selecting the Large-Volume protocol, ensure that 400 $\mu$ l of MTL buffer has been added to the sample(s) prior to loading on the instrument.

8. Follow the instructions displayed on the screen to set up the reagent cartridges, digest tubes, tip holders, and elution tubes.

**Note:** Ensure that the reagent cartridges snap into place in the cartridge rack and that the caps are removed from the sample tubes and elution tubes prior to loading on the deck. The caps from the sample tubes may be discarded. The caps from the elution tubes are retained and replaced upon completion of the protocol.

9. If fewer than 14 samples are being extracted, reagent cartridges and consumables need to be used only for the occupied channels.
10. Upon completion of the protocol, remove the elution tubes containing the purified DNA and cap the tubes.
11. The extracted DNA is now ready for quantification (optional for known samples) and/or amplification. DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be transferred to -20°C for long-term storage.

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12. Clean the instrument by wiping down with ethanol, followed by distilled water, after each use. Do NOT use bleach, as it may react with the extraction reagents.
  
13. Clean the piercing unit after each use by selecting option 2 ("Man") on the main menu, then option 3 ("Clean"). Then wipe each piercing unit down with ethanol.
  
14. Refer to EZ1 Advanced XL Maintenance Work Instructions section of this document for UV decontamination procedures.

### 3.1.3 Casework Samples – Differential Extraction

1. Prepare the 1:1 diluted G2 buffer:
  - (Number of samples + 3) x 1040µl G2 buffer
  - (Number of samples + 3) x 1040µl distilled water
  
2. Cut a portion of the sample and place in a 2.0ml tube.
  
3. Add 600µl of sterile de-ionized water to each of the tubes.
  
4. Vortex briefly. Incubate overnight at 37°C. Pre-soaks/water soaks with less incubation time shall be performed only with documented pre-approval of the Forensic Biology Supervisor or DNA Technical Manager after evaluation of the sample.
  
5. Agitate the samples for 1-2 minutes by vortexing or using a sterile pipette tip or a sterile toothpick to remove the cells from the substrate.
  
6. For each sample, transfer the substrate to a spin basket and place the basket back in the sample tube. Centrifuge the sample tubes for 5 minutes at 10,000 to 14,500 RPM.
  
7. Transfer approximately 550µl of the supernatant to a new, labeled 2.0mL tube. Also transfer the spin basket containing the substrate. Retain until the case has been completed.

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8. Re-suspend the cell pellet and spot 3µl on a microscope slide. Stain and grade the slide.
9. If sperm and epithelial cells are observed proceed to step 10.

**OPTIONS:**

- If few/no sperm/epithelial cells are observed, a second extraction may be performed by adding more of the original evidence item to the sample tube and repeating steps 2-8.
  - Alternatively, the substrate may be added back to the sample tube for digestion or it may be digested separately.
    - If adding the substrate back to the sample tube, place substrate back in the tube and proceed to step 10.
    - If digesting separately, place substrate into a new 1.5mL tube and label it as "Q#sub"; analysis of the substrate resumes at step 17.
  - When no sperm are observed, it is not necessary to divide the sample into separate epithelial and sperm fractions (document on the DNA worksheet). If not separating the sample, add up to 390µl of 1:1 diluted G2 buffer + 10µl of Proteinase K solution and proceed to step 18.
  - When no NECs are observed, it is not necessary to divide the sample into separate fractions (document on the DNA worksheet). Add 190µl of 1:1 diluted G2 buffer + 10µl Proteinase K solution + 10µl DTT and proceed to step 18.
10. To the re-suspended cell pellet (may include substrate if added back), add up to 390µl of 1:1 diluted G2 buffer + 10µl of Proteinase K solution.
  11. Vortex and spin briefly to force the material into the extraction fluid.
  12. Incubate for at least two hours at 56°C. During the incubation step label a new sterile tube for each sample, including the reagent blank. Label these as the epithelial fractions.

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13. Centrifuge the sample tubes for 5 minutes at 10,000 to 14,500 RPM. If the substrate was added back to the tube (step 9 option), using a sterile toothpick to place the substrate into a spin basket and centrifuge for 5 minutes at 10,000 to 14,500 RPM. Remove all but 50µl of the supernatant and transfer to the epithelial fraction tube. Analysis of the epithelial fraction resumes at step 19.

**Optional:** At this stage, the analyst may perform a second epithelial cell digest (repeat steps 10-13) on samples with a large number of nucleated epithelial cells. This is called a “double digest” and should be documented on the DNA worksheet and on the Standards and controls worksheet for the accompanying reagent blank. This may be based on the initial microscopic examination or on a second examination.

14. Wash the sperm pellet by adding approximately 500µl of dilute G2 buffer. Vortex briefly and centrifuge at approximately 10,500 to 14,000 rpm for 5 minutes.
15. Remove and discard the supernatant.
16. Repeat the wash two more times for a total of three washes. If few sperm were observed, the number of washes for the sperm fraction may be decreased.
17. Add 190µl of 1:1 diluted G2 buffer + 10µl Proteinase K solution + 10µl DTT.

**Note:** If the substrate was digested separately, the volume of diluted G2 buffer may be increased to account for absorption by the substrate.

18. Vortex and spin briefly to force the material into the extraction fluid.
19. Incubate overnight at 56°C.

- If the reagent cartridges are being stored between 2°C and 8°C, place them at room temperature overnight. Alternatively, they may be warmed in a 37°C incubator for approximately 1 hour.

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20. Insert the DNA Investigator card into the slot located on the front of the BioRobot EZ1 Advanced-XL.
21. Turn on the EZ1 power switch on the back of the instrument.
22. Directions are displayed on the screen on the front of the instrument. Press the START button to select a protocol (Trace or Large-Volume), elution buffer and elution volume.

**Note:** If selecting the Large-Volume protocol, ensure that 400  $\mu$ l of MTL buffer has been added to the sample(s) prior to loading on the instrument.

23. Follow the instructions displayed on the screen to set up the reagent cartridges, digest tubes, tip holders, and elution tubes.

**Note:** Ensure that the reagent cartridges snap into place in the cartridge rack and that the caps are removed from the sample tubes and elution tubes prior to loading on the deck. The caps from the sample tubes may be discarded. The caps from the elution tubes are retained and replaced upon completion of the protocol.

**Note:** If fewer than 14 samples are being extracted, reagent cartridges and consumables need to be used only for the occupied channels.

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24. For sperm fractions:

- Transfer 200 $\mu$ l of the digest to a 1.5mL screw cap tube, add 1 $\mu$ l carrier RNA solution, run Trace protocol and elute in 50 $\mu$ l to 200 $\mu$ l TE buffer.

For epithelial fractions:

- Transfer 200 $\mu$ l of the digest to a 1.5mL screw cap tube, add 1 $\mu$ l carrier RNA solution, run Trace protocol and elute in 50 $\mu$ l to 200 $\mu$ l TE buffer.

**Note:** If necessary, prepare the carrier RNA solution by reconstituting the carrier RNA in 310 $\mu$ l of sterile, de-ionized water. Vortex and spin briefly. Prepare 20 $\mu$ l, single use, aliquots in 0.5mL tubes and store at -20°C.

Options for fractions with none/few/1+ cells and samples not separated:

**Option 1 – Large-Volume Protocol:**

- *For samples that typically yield less DNA or if the sample required additional digest buffer for thorough cell lysis, the analyst may transfer up to 500 $\mu$ l of the digest solution to an EZ1 sample tube, add 1 $\mu$ l carrier RNA solution, and add 400 $\mu$ l of Buffer MTL to each tube(s).*
- *Load the sample(s) onto the EZ1, select the Large-Volume Protocol, and elute in 50 $\mu$ l TE buffer. An additional reagent blank with Buffer MTL added must be extracted with all Large-Volume Protocol samples.*

**Option 2 – Large-Volume Protocol for Consumed Samples:**

- *If the entire evidence sample has been consumed and digested, the analyst shall transfer the entire volume of digest solution to an EZ1 sample tube (or tubes – with no more than 500 $\mu$ l in each tube), add 1 $\mu$ l of carrier RNA solution and add 400 $\mu$ l of Buffer MTL to the tube(s).*
- *Run the Large-Volume protocol and elute in 100 $\mu$ l TE buffer.*

**NOTE:** *If any sample is extracted using the Large-Volume protocol and eluted in 50 $\mu$ l, then the reagent blank accompanying that sample will suffice as the reagent blank for a consumed sample extracted using the Large-Volume protocol and eluted 100 $\mu$ l. If no other Large-Volume protocol samples are extracted, ensure that a reagent blank is extracted using the Large-Volume protocol and eluted in at least 50 $\mu$ l.*

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25. Upon completion of the EZ1 protocol, remove and cap the elution tubes containing the purified DNA.
26. The extracted DNA is now ready for quantification and amplification. DNA extracts can be stored for several weeks at 2°C to 8°C. Extracts should be transferred to -20°C for long-term storage.
27. Clean the instrument by wiping down with ethanol, followed by distilled water, after each use. Do NOT use bleach, as it may react with the extraction reagents.
28. Clean the piercing unit after each use by selecting option 2 ("Main") on the main menu, then option 3 ("Clean"). Then wipe each piercing unit down with ethanol.
29. Refer to EZ1 Advanced XL Maintenance Work Instructions section of this document for UV decontamination procedures.

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#### **Section 4 DNA Quantification**

*As mandated by the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories, all forensic questioned samples must be quantified prior to amplification. Quantification is not mandatory for database samples or forensic known samples.*

References: Quantifiler® Kits User's Manual and Mx3000P On-line User's Manual (<http://www.stratagene.com/manuals/>)

#### **4.1 Quantification of DNA using AB Quantifiler™ Kit on Stratagene® Mx3000/3005P™**

##### **4.1.1 Preparing the Mx3000P™ for a Run**

- Open the MxPro-Mx3000P™ Software.
- In the New Options pop up select Quantitative PCR(Multiple Standards) and ensure the box Turn lamp on for warm-up? Is checked. Select OK.
- Click File>Open and navigate to QPCR-template.mxp which is located in DNA\_Share in the Mx3000P Data Storage file.
- Save the file with the run date and analyst's initials (i.e. QPCR-YY-MMDDKAH) in the appropriate analyst folder.
- Select the appropriate well types using the scroll down bar labeled Well type on the right hand side of the screen. Choose Unknown for all samples irrespective of type (questions or knowns) and choose NTC (no template control) for the wells with no template control. Two wells of each plate are to be run as NTCs. These wells contain only master mix. The first two columns always contain the standard curve.

**Note:** The lamp takes twenty minutes to warm-up therefore open the Mx3000P software before sample preparation, to allow time for lamp to warm-up. On the bottom right of the screen a box will indicate when the lamp is ready.

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#### 4.1.2 Preparation of DNA Quantitation Standards

On the bench top prepare a three-fold serial dilution of the Quantifiler™ Human DNA Standard (provided in kit) in sterile H<sub>2</sub>O as follows:

- Label eight sterile 0.5mL microcentrifuge tubes A through H.
- Add 30 $\mu$ L sterile H<sub>2</sub>O to tube A.
- Add 20 $\mu$ L sterile H<sub>2</sub>O to tubes B through H.
- Thaw the Quantifiler™ Human DNA Standard completely. Vortex for 3 to 5 seconds and spin briefly.
- Transfer 10 $\mu$ L of DNA Standard (200ng/ $\mu$ L stock) into tube A. Vortex and spin briefly.
- Transfer 10 $\mu$ L of prepared Standard A into tube B. Vortex and spin briefly.
- Continue the serial dilution through tube H.

The approximate quantities of DNA in prepared Standards A through H are as follows:

Standard A  $\approx$  50ng/ $\mu$ L  
Standard B  $\approx$  16.7ng/ $\mu$ L  
Standard C  $\approx$  5.56ng/ $\mu$ L  
Standard D  $\approx$  1.85ng/ $\mu$ L  
Standard E  $\approx$  0.62ng/ $\mu$ L  
Standard F  $\approx$  0.21ng/ $\mu$ L  
Standard G  $\approx$  0.068ng/ $\mu$ L  
Standard H  $\approx$  0.023ng/ $\mu$ L

**Note:** In-house experiments have demonstrated that the standard curve is stable for at least one week and should be stored in the freezer with documentation of the date made, expiration date and the H<sub>2</sub>O lot number.

#### 4.1.3 Preparing the Reactions

Calculate the volume of each component needed to prepare the reactions in duplicate.

- Quantifiler™ Human Primer Mix at 10.5 $\mu$ L per reaction.
- Quantifiler™ PCR Reaction Mix at 12.5 $\mu$ L per reaction.

**Note:** Include three additional reactions in your calculations for every sixteen samples prepared, to provide excess volume for loss that occurs during reagent transfers. A designated 96-well plate QPCR worksheet is to be used for well mapping. Questioned samples will be run in duplicate. Known samples, if quantified, need not be run in duplicate.

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- Thaw the primer mix completely, then vortex 3 to 5 seconds and centrifuge briefly before opening the tube.
- Swirl the PCR Reaction Mix gently before using. DO NOT vortex.
- Pipette the required volumes of components into an appropriately sized tube.
- Vortex the master mix for 3 to 5 seconds.
- Place a new Stratagene®, or equivalent, 96 well plate into well plate working rack.

**Note:** Stratagene® 96-well plates are different than the plates used for amplification.

- Dispense 23 $\mu$ L of the PCR master mix into each reaction well of the 96-well plate.
- Add 2 $\mu$ L of sample or standard to the appropriate wells. No sample is added to the NTC wells.
- Use powder-free gloves and a Kimwipe to cover wells with Stratagene®, or equivalent, optical strip caps.
- Apply downward force to each cap then view tray from side to ensure all caps are seated evenly.
- Centrifuge samples in a plate centrifuge to make sure there are no bubbles in the wells.

### 4.1.4 Sample Loading

- Use care when loading and unloading the 96 well plate; the thermal block can be hot.
- Verify that the status LED (upper LED on the instrument) is solidly lit, indicating that the instrument is ready for use.
- Open the door of the Mx3000/3005P™, located at the front of the instrument.
- To gain access to the thermal block, unlatch the hot-top assembly by pulling forward on the handle and then lifting the hot-top up and away from the thermal block.
- Insert the 96 well plate into the thermal block. (Plate goes in with the 'A' well in the upper left)
- Close hot-top and door on the instrument.

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### 4.1.5 Starting the Run

- Confirm that the worksheet has been saved as previously described.
- Click on the Run icon in the upper right hand corner of the screen. The run status box will appear.
- Check the Turn lamp off at end of run box if this will be the last run of the day.
- Click Start.

### 4.1.6 Analyzing the Data

- When the run is complete, click the Analysis icon in the upper right hand corner of the screen.
- You are now in the Analysis Selection/Setup screen. Make sure that all used sample wells are highlighted. Clicking All in the upper left hand corner of the 96-well plate map will select/de-select all 96 wells at once. If you have fewer than 96 samples, individual well or columns can be individually selected by holding down the ctrl key while selecting the wells.
- On the right hand side, under Algorithm enhancements, click off the Moving average and Amplification based threshold boxes leaving only the Adaptive baseline box checked.
- Click on the Results tab at the top of the 96-well plate map.

#### 4.1.6.1 Checking the Standard Curve

- Under Well types shown in the lower right hand corner of the window ensure Standard is the only option selected.
- In the Area to analyze box select Amplification plots and in the Assays shown box in the lower left hand corner of the window ensure FAM is the only one selected by deselecting ROX, HEX, and CY5.
- Check the Ct values of the standards in the box at the lower right hand corner of the screen. If a standard fails to amplify (no Ct value) or if there is a large variation in the Ct value of the duplicate standards, you can remove the data point for that well. This is done by returning to the Analysis Selection/Setup screen and de-selecting the well. When returning to Results, the curve will automatically be re-calculated.

**Note:** No more than 3 points should be taken out and at least one of the high and low points (A and H) should always be retained.

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- Next, in the Area to analyze box in the upper right of the screen select the Standard curve option. Under Assays shown (lower left) make sure FAM is the only dye selected. **Print this view.**
  - $R^2 \geq 0.99$ . If  $< 0.98$  see Quantifiler™ Kits User's Manual (p. 5-4).
  - Slope Range  $-2.9$  to  $-3.3 \pm 0.3$
  - Efficiency Range 85% to 115%
- Generally, the Ct value of prepared Standard A should lie between 22 and 23 and the Ct value of prepared Standard H should lie between 32 and 33. When the standard curve shifts to the right (higher Ct values) a higher amount of template DNA should be targeted for STR analysis. When the standard curve shifts to the left (lower Ct values) a lower amount of template DNA should be targeted for STR analysis. How much of an adjustment needs to be made will depend on the degree of shift of the standard curve.

**Note:** If the standard curve plot values are marginally outside the given ranges, the data may be acceptable at the discretion of the Technical Manager.

#### 4.1.6.2 Checking the Internal PCR Control (IPC)

- In the Area to analyze box select Amplification plots. Under Assays shown (lower left), select only IFX to view the Internal PCR Control (IPC). Select the Standard, Unknown and NTC boxes in the Well types shown box in the lower right hand corner of the window.
- Check all IPC Ct values (found in lower right hand box). The values should be between 20 and 30.
  - A high Ct value or no Ct for the IPC can indicate inhibition or competition between extremely high concentrations of human genomic DNA.
  - If a sample has a Ct value  $>30$ , make a note of this so you can account for possible inhibition. This will need to be considered when setting up for STR amplification.
  - If a sample has a low IPC Ct value or no IPC Ct, but a high initial template quantity, it is unlikely that PCR inhibitors are present.
  - If a sample has no IPC Ct and no initial template quantity, it is not possible to distinguish between the absence of DNA and PCR inhibition.
  - Document the average IPC Ct value for all unknown samples on the Initial Template Quantity printout

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**4.1.6.3 Checking the Passive Reference**

Check the passive reference for background noise by only selecting the ROX in the Assays shown box in the lower left corner of the screen. If the ROX baseline appears elevated or jagged, refer to the Quantifiler® Kit User's Manual.

**4.1.6.4 Checking the No Template Controls (NTC)**

- Check the NTCs by only selecting the NTC box in the Well type shown on the bottom right of the window and select both FAM and HEX on the bottom left in the Assays shown box.
- Make sure the HEX Ct value is between 20 and 30 and the FAM has no Ct value.
- HEX and FAM Ct values other than these could indicate contamination of the master mix. Consult with the DNA Technical Manager.

**4.1.6.5 Checking the Initial Template Quantity**

- On the right hand side of the screen under Area to analyze select Initial template quantity and in Assays shown select FAM only. **Print this screen** to show concentration of DNA (ng/ $\mu$ L).
- From this view you can determine the amount of sample to amplify for STR analysis.
  - Reagent blanks should give no value for initial template quantity.
  - Very low initial template quantity values could be the result of dust or debris interfering with the optical path. All blanks will be amplified regardless of the quantification result. Therefore, the quantification result alone should not be used to assess contamination of a blank.

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**Section 5 DNA Amplification**

**5.1 Amplification Set-up of Forensic Casework Samples**

1. Allow the samples to warm to room temperature, then vortex and spin briefly in a microcentrifuge. Samples that have been stored at 2-8°C or frozen for a week or more may be warmed in an incubator to bring them to room temperature. These samples should be vortexed longer to optimize recovery.
2. Transfer the amplification reagents to the designated PCR set-up area. Place the amplification reagent tubes in a rack that is dedicated to PCR set-up.

**Note:** Do not expose reagents to light for extended periods of time.

3. Obtain a 96-well amplification plate.
4. Ensure that all kit components have thawed completely before use. Vortex reagents and centrifuge briefly to ensure uniform mixing and collection of tube contents.

**Note:** Centrifugation of the primer mix should be minimal to avoid primer collecting at the bottom of the tube.

5. Prepare a PCR master mix by adding the following volumes of reagents to an appropriately sized tube:
  - # of samples x 2.5µL Gold STR 10X Buffer
  - # of samples x 2.5µL PowerPlex® 16 10X Primer Pair Mix
  - # of samples x 0.8µL AmpliTaq Gold™ DNA Polymerase (5U/µL)

**Note:** Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the DNA Amplification/electrophoresis worksheet.

6. Vortex the master mix and spin briefly. Transfer 5.8µL of master mix to each sample well. Cover the entire well plate with Glad® Press 'n Seal (or equivalent).

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7. Prepare the samples to be amplified as follows (in individual 0.5mL tubes):

Forensic Case Samples

Add approximately 0.8ng to 2.0ng template DNA. The amount of template DNA may be higher or lower depending on the sample (i.e. low-level samples that are likely to be mixtures may be targeted higher, while bloodstains likely to be single-source may be targeted lower). Add sterile de-ionized water to bring the sample to a final volume of 19.2µL.

Quantification Value <0.05ng/µl

Samples with a quantification value <0.05ng/µl (or PCR target template estimate of less than 0.95ng) shall be routinely amplified in duplicate to ascertain reproducibility of alleles above and below 100RFU. Data interpretation and reporting of conclusion(s) will depend on whether the profile indicates a single source or mixed DNA sample.

Quantification Value >0.05ng/µl

Samples with a quantification value >0.05ng/µl will NOT be routinely amplified in duplicate. If the profile generated includes artifacts (e.g. stutter/true allele) and/or ambiguous allele peaks, the sample may need to be re-amplified to verify reproducibility of the data.

Negative Quantification Value

Samples with no detectable DNA, including reagent blanks and negative controls, may be amplified one time (i.e. duplicate amplification not required).

**Note:** When amplifying greater than 10µL of template DNA, be aware that inhibitors that were not detected during the quantification step may interfere with amplification.

Reagent Blank Sample(s)

Add 19.2µL of the extraction reagent blank.

Positive Control Sample

Vortex and spin the tube briefly and add 5µL of the diluted 9947A Control DNA (or other positive control DNA supplied by the PCR kit vendor) to a 0.5mL tube containing 14.2µL of sterile, de-ionized water.

**Note:** Over extended periods of storage of the positive control, it may be necessary to increase the volume of 9947A and decrease the volume of water to achieve the required concentration of the sample.

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Negative Control Sample

Add 19.2µL of sterile de-ionized water.

8. Transfer the entire 19.2µL of the above prepared samples to the appropriate sample well, already containing the PCR master mix. During sample addition, the pipette tip is inserted through the Press 'n Seal.
9. Once all samples have been added, remove the Press 'n Seal and cover the plate with a sheet of amplification tape.
10. Transfer the plate to the PCR room and place directly into the thermal cycler. Cover well plate with a silicone spacer (if needed) prior to closing the thermal cycler cover. Start the run.
11. Store amplified products at 2-8°C. All amplified products will be disposed of upon completion of the technical and administrative reviews of the case.

**5.2 Amplification Set-up of Offender Database Samples**

1. Allow the samples to warm to room temperature, then vortex and spin briefly in a microcentrifuge. Samples that have been stored at 2-8°C or frozen for a week or more may be warmed in an incubator to bring them to room temperature. These samples should be vortexed longer to optimize recovery.
2. Transfer the amplification reagents to the designated PCR set-up area. Place the amplification reagent tubes in a rack that is dedicated to PCR set-up.

**Note:** Do not expose reagents to light for extended periods of time.

3. Obtain a 96-well amplification plate.
4. Ensure that all kit components have thawed completely before use. Vortex reagents and centrifuge briefly to ensure uniform mixing and collection of tube contents.

**Note:** Centrifugation of the primer mix should be minimal to avoid primer collecting at the bottom of the tube.

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5. Prepare a PCR master mix by adding the following volumes of reagents to an appropriately sized tube:
  - (# of samples + 6) x 2.5 $\mu$ L Gold STR 10X Buffer
  - (# of samples + 6) x 2.5 $\mu$ L PowerPlex® 16 10X Primer Pair Mix
  - (# of samples + 6) x 0.8 $\mu$ L AmpliTaq Gold™ DNA Polymerase (5U/ $\mu$ L)

**Note:** The volumes are calculated automatically on the Database Batch Worksheet.

6. Vortex the master mix and spin briefly, when possible. Transfer 5.8 $\mu$ L of master mix to the sample wells for the amplification positive and negative controls and the extraction reagent blank(s).
7. Add the following volume of sterile, de-ionized water to the master mix:  
(# of samples + 2) x 17.2 $\mu$ L

**Note:** This volume is calculated automatically on the Database Batch Worksheet.

8. Vortex the master mix and spin briefly. Transfer 23 $\mu$ L of master mix to the remaining sample wells.
9. Cover the well plate with Glad® Press 'n Seal film (or similar product). During sample addition, the pipette tip is inserted by puncturing through the Press 'n Seal film. This will aid in tracking the sample wells/tubes which have sample already transferred to them and minimize the chance of well to well contamination.
10. Prepare the positive and negative amplification controls and reagent blank as described below:

Reagent Blank Sample(s)

Add 19.2 $\mu$ L of the extraction reagent blank(s) to the appropriate well(s).

Positive Control Sample

Vortex and spin the tube briefly and add 5 $\mu$ L of the diluted 9947A Control DNA (or other positive control DNA supplied by the PCR kit vendor) to a 0.5mL tube containing 14.2 $\mu$ L of sterile, de-ionized water. Transfer the entire 19.2 $\mu$ L to the appropriate well.

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**Note:** Over extended periods of storage of the positive control, it may be necessary to increase the volume of 9947A and decrease the volume of water to achieve the required concentration of the sample.

Negative Control Sample

Add 19.2 $\mu$ L of sterile, de-ionized water to the appropriate well.

Database Sample(s)

Transfer 2 $\mu$ L of each offender extract to the appropriate well or tube.

11. Once all samples have been added, remove the Press n Seal and cover the plate with a sheet of amplification tape.
12. Transfer the plate to the PCR room and place directly into the thermal cycler. Cover well plate with a silicone spacer (if needed) prior to closing the thermal cycler cover. Start the run.
13. Store amplified products at 2-8°C. All amplified product will be disposed of upon completion of the reviews and upload of the batch.

**Note:**

Re-amplification Samples

It may be necessary to adjust the volume of water (and of offender DNA extract) for re-amplifications or when processing older samples. The total volume, per sample, should always equal 19.2 $\mu$ L.

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## Section 6 Capillary Electrophoresis

### 6.1 Applied Biosystems 3130xl Genetic Analyzer

#### 6.1.1 Start the Software and Instrument

- Turn the computer on and log into the computer.
- Turn on the 3130xl Genetic Analyzer. Wait for the green status light to appear.
- Launch the Data Collection Software.  
**Start > All Programs > Applied Biosystems > Data Collection > Run 3130xl Data Collection v3.0**
- The service console displays all of the applications running. When all squares are green, all the applications are running. This could take several minutes.

**Note:** When shutting down the Data Collection Software use the service console by clicking the stop all button. For detailed start up and shut down procedures see the Applied Biosystems 3130/3130xl Genetic Analyzers Quick Reference Card.

#### 6.1.2 Preparing the Instrument

Refer to the 3130xl Instrument Maintenance Work Instructions section of this document if any maintenance or instrument preparations are required prior to running samples on the instrument.

#### 6.1.3 Sample Preparation for the 3130xl

Internal Lane Standard 600 (ILS 600) is included with the PowerPlex® 16 System as the internal lane standard for four color detection and analysis of amplified samples.

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
  - # of samples x 1µL ILS 600
  - # of samples x 9µL Hi-Di™ Formamide

Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on each of the electrophoresis worksheets.

- Vortex the master mix and spin briefly. Transfer 10µL of master mix to the appropriate sample wells of a 96 well plate.
- Add 10µL of Hi-Di™ Formamide to unused wells of a set of 16. (i.e. A1...H2).

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- Add 1µL of allelic ladder and up to 3µL of each amplified sample (reagent and amplification blank volumes must correspond to the largest volume of amplified sample added to the plate) to the appropriate wells. At least one ladder must be contained within each injection of 16 samples. When all samples have been added, cover with a plate septa.

**Note:** Instrument detection limits vary. Therefore, injection time or the amount of ladder/amplified product may need to be increased or decreased. The interpretation of data is discussed in greater detail in the Forensic Biology Procedures Manual.

- Brief centrifugation of the 96 well plates will remove bubbles that may affect analysis.
- Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument.
- Align the septa with the plate retainer and snap the retainer onto the reaction plate and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- To place the plate assembly into the instrument:
  - Press the tray button on the front of the instrument and wait for the autosampler to stop moving.
  - Open the front doors.
  - Place the plate assembly on the autosampler in position A or B.
  - There is only one proper orientation for the plate, with the notched end of the plate base in the lower right corner.
  - Ensure the plate assembly lies flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off the autosampler. Once the plate is in place the position of the plate turns to yellow in the Run Scheduler view on the monitor.
  - Close the instrument doors.

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## 6.1.4 Creating a Plate Record

### 6.1.4.1 Create a Plate Record from the Data Collection Software

- Navigate to the following:  
**GA instruments > ga3130xl > Plate Manager**
- Click New and complete the New Plate Dialog Box
  - Enter a name for the plate.
  - Casework samples
  - Batch Number initials\_Q/K\_date plate set-up (i.e. 11-0103CMD\_Q\_110130)
  - Database samples
  - DB Batch Number initials (i.e. DB11-0314AB\_KAL) this may vary depending on the analyst.
  - A description for the plate record is optional.
  - In the Application drop-down list, select **GeneMapper-Generic**
  - In the Plate type drop-down list, select **16-well**
  - Enter your own initials for the owner and the operator.
  - Click **OK** and the GeneMapper Plate Editor opens.
- In the Sample Name column of a row, enter sample name and/or sample code (invalid characters for naming are / \ \* ? < > space).
- In the Comment column, enter any additional comments or notations for the sample.
- Leave the following columns blank: Sample Type, Size Standard, Panel, Analysis Method and Snp Set.
- Text can be entered for User-Defined columns 1 to 3 or they can be left blank.
- In the Results Group 1 column, select the analyst's initials from the drop-down list.
- In the Instrument Protocol 1 column, select a protocol from the drop-down list. The protocols for PowerPlex 16 start with PP16\_3kV\_ and have options of 3sec, 5sec, 8sec, and 10sec injections.
- If samples are to be injected more than once:
  - Select Edit > Add Sample Run. Additional Results Group and Instrument Protocol columns are added to the right end of the plate record. This can be done before, during, or after the run.
  - Complete the columns for the additional run(s)
  - Click **OK** to save and close the plate record.

**Note:** After clicking OK within the Plate Editor, the completed plate record is stored in the Plate Manager database. Once in the Plate Manager database, the plate record can be searched for, edited, exported, or deleted.

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#### 6.1.4.2 Create a Plate Record from an Export File

- Open a plate record template export file (i.e. .txt exported from instrument).
- Copy and paste sample name and/or sample code info into the export file. Ensure all spaces after sample name have been deleted. (Rows not in use may be deleted but do not delete any columns.) Save the record.
- Using the 3130xl Data Collection Software navigate to the following:  
**GA instruments > ga3130xl > Plate Manager**
- Click **Import** and navigate to the saved plate record.
- Click **OK** to save and close the plate record.

#### 6.1.5 Linking a Plate and Starting a Run

- In the Tree pane of the Data Collection Software  
**GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run Scheduler**
- Click **Find All**. All plates in the database will display in plate record section. (You may also type the name of the plate in the box **Scan or Type Plate ID** to pull up plate.)
- Select the plate record of the plate to be run, then click the plate position indicator that corresponds to the plate you are linking. The plate position indicator will be yellow if there is a plate in that position of the autosampler. The indicator will turn green once you click to link the plate.
- Confirm that your data has a valid export path by performing the following:
  - Navigate to **GA instruments > Results Group**
  - Double click results group to be used.
  - Click **Destination** tab.
  - Click **Test**. Verify whether or not the test succeeded and the file path was found.
- To review the run schedule before beginning the run navigate to the following:  
**GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run scheduler > Run View**
- Once a plate is linked, the green run arrow will turn green. Click the green arrow in the toolbar.
- A dialog box stating "You are about to start processing plates" click **OK**.

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- The software automatically performs a run validation:
  - If the validation passes, the run starts
  - If any of the validation tests fails, the run will not start. Refer to the Applied Biosystems 3130/3130xl Genetic Analyzer's Maintenance, Troubleshooting, and Reference Guide.

### 6.1.6 Viewing Data During the Run

- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run scheduler > Plate View**
  - This view shows a list of plates in the database and which plate is linked.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run scheduler > Run View**
  - This view shows a list of all the runs scheduled. If they are validated, collecting data, or completed. It also shows where each run is located on the 96-well plate.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Instrument Status**
  - This view tells you the System status, EP voltage, EP current, Laser Power, Laser Current, Oven Temperature, array and polymer information.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Instrument Status > Event Log**
  - This view shows the event log and error log.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Capillaries Viewer**
  - Select the checkboxes of the capillaries for which you want electropherograms displayed. As more capillaries are selected, the refresh rate becomes slower. The view can be zoomed in and out using the magnifying glass icons in the toolbar.
- **GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Cap/Array Viewer**
  - This window is used during a run to examine the quality of the data, which is displayed as color data for the entire capillary array. All the capillaries are viewed similar to a gel view.

**Note:** Do not leave the computer on these last two windows for extended periods of time due to large amounts of memory needed to view the data.

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**6.2 Applied Biosystems 3500xl Genetic Analyzer**

**6.2.1 Start the Software and Instrument**

- Turn the computer on and log into the computer
- Turn on the 3500xl Genetic Analyzer. Wait for the green status light to turn on.
- Ensure both the Daemon and Service Monitor has started by observing a green checkmark icon in the lower right hand corner. This indicates that all 3500 services have loaded. This may take several minutes.
- Launch the Data Collection Software:  
**Start > Programs > Applied Biosystems > 3500**
- Log in to the 3500 Series Data Collection Software

**6.2.2 Preparing the Instrument**

- Navigate to the Dashboard of the software
- Check consumables by clicking **Refresh** to update consumable status.
- Refer to the 3500xl Instrument Maintenance Work Instructions section of this document if any maintenance or instrument preparations are required prior to running samples on the instrument
- Click **Start Pre-heat** to warm the oven.
- Check the pump assembly for bubbles and run the Remove Bubble wizard if necessary.

**6.2.3 Sample Preparation for the 3500xl**

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
  - # of samples x 0.5µl ILS 600
  - # of samples x 9.5µl of Hi-Di Formamide

**Note:** Prepare enough for a few extra reactions to allow for loss during pipetting. This is calculated automatically on the DB-BlankWkst3500xl Reagent Worksheet.

- Vortex the master mix and spin briefly. Transfer 10µl of master mix to appropriate sample wells of a 96 well plate.
- Add 10µl of Hi-Di Formamide to unused wells of a set of 24 (i.e. A1-H3).
- Add 1µl of allelic ladder and up to 2µl of each amplified sample to the appropriate wells. Reagent and amplification blank volumes must correspond to the largest volume of amplified sample added to the plate. When all samples have been added, cover with plate septa.

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- Briefly centrifuge the 96 well plate to remove any bubbles. Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.

## 6.2.4 Creating a Plate Record

### 6.2.4.1 Create a Plate Record from the Data Collection Software

- In the dashboard click **Create New Plate**
- In the Define Plate Properties screen:
  - Enter a plate name (i.e. DB10-1227AB\_KAL), this may vary depending on analyst
  - Select 96 for the number of wells
  - Select HID for plate type
  - Select 36cm for capillary length
  - Choose POP4 for polymer
  - Owner, barcode and description are optional fields.
- Click **Save Plate**
- Click **Assign Plate Contents**
- Using either plate view or table view enter sample names in the corresponding wells.
- Under the heading Assays, File Name Conventions, and Results Groups click **Add from Library** select one of the following Assays:
  - Pplex16\_15sec
  - Pplex16\_20sec
  - Pplex16\_24sec
- Select a naming convention, and a results group of your choice. Click **add to plate** and then click **close**.
- Select the wells for which to specify the assay file name conventions and results groups and enable the checkbox next to the name to assign it to the selected wells.
- Click **Save Plate** and save.

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### 6.2.4.2 Create a Plate Record from an Export File

- Open a plate record template export file (i.e. .xls exported from instrument).
- Copy and paste sample name info into the export file. (Rows not in use may be deleted but do not delete any columns) Save the record.
- Using the 3500xl Data Collection Software, click on **Create New Plate** on the dashboard.
- In the Define Plate Properties screen:
  - Enter a plate name (i.e. DB10-1222AB\_KAL)
  - Select 96 for the number of wells
  - Select HID for plate type
  - Select 36cm for capillary length
  - Choose POP4 for polymer
  - Owner, barcode and description are optional fields.
- Click **Save Plate**.
- Click **Assign Plate Contents**.
- Click **Import** at the top of the screen and navigate to the saved plate record (make sure .xls files are searched).
- Click **Save Plate** and save.

### 6.2.5 Linking a Plate and Starting a Run

- Click **Link Plate for Run**. A message will appear saying Plate loaded successfully. Click **OK**.
- Ensure plate is linked to proper position on 3500xl.
- Select **Create Injection List**.
- Click **Start Run**.

**Note:** Ensure all prompts have cleared and the run begins before walking away.

### 6.2.6 Viewing Data During a Run

Refer to Applied Biosystems 3500/3500xl Genetic Analyzer User Guide

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**Section 7 Data Analysis with GeneMapper ID-X**

References: *GeneMapper™ ID-X Software User Guide*  
*GeneMapper™ ID-X Software Tutorial*  
*GeneMapper™ ID-X Software User Bulletin*

**7.1 Analysis of Casework Data with GeneMapper™ ID-X**

**7.1.1 Logging in to GeneMapper™ ID-X**

- Open GeneMapper™ ID-X
- Select the User Name and Database Host from the drop-down list and enter the appropriate password.
- Click OK.
- The main project window will open.

**3.7.S Creating a Project**

- To add samples from the collection software at a workstation, go to the edit menu and select **Add Samples to Project**. Navigate to **My Computer > Uncontrolled Documents > Section Shares > DNA Share > Data** on the laboratory "I Drive" as per current designation(s).

**Note:** Location is dependent on network mapping and may vary slightly.

- Select the raw data folder to be imported or select individual samples and click **Add to list**.
- When all samples have been selected, click **Add**.
- Select the appropriate Sample Type, Analysis Method, Panel and Size Standard for each sample and click the green arrow on the tool bar to analyze the samples.

**Note:** Ensure that the appropriate analysis parameters (casework or database) are selected. Casework (not database) analysis parameters are selected. Reagent blanks and negative controls are analyzed with a **Blank\_Method**.

- The user will be prompted to name and save the project to the appropriate Security Group (GeneMapper ID-X Security Group). Name the project with the Batch name, Q or K, the run date (YYMMDD) and the injection time. Click OK.
  - Example: 05-0204JMS\_Q\_050204\_5sec

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- Analysis is complete when the green arrows in the Status column on the left are gone.

### 3.7.S Viewing the Data

- Check your ILS by highlighting all samples and clicking on the Size Match Editor, the icon with the red peaks on the toolbar. Make sure all samples have ILS peaks sized correctly. Database analysis will include notes on the Sizing Quality (SQ<0.9) of the ILS. Casework analysis will include similar notes if and when deemed necessary. Refer to the Data Interpretation section of the Forensic Biology Procedures for more information.
- To view each sample, highlight the sample and click on the icon with the colored peaks to display plots. (This can also be done by View > display plots or Ctrl + L).
- The electropherogram can be printed from the sample's plot window. Various plots have been created for different sample types. Commonly used plot displays include: Traditional Genotype Plot, Casework Blank, Casework artifacts and Casework zoom view.
- To view information on a sample's injection time and other run information, highlight the sample of interest and click **View** on the toolbar > Sample Info (Ctrl + F1).
- To view raw data for a sample, click **View > Raw Data**.
- To view allele calls by sample and locus click the Genotypes tab from the main project window.

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**Section 8 Statistical Analysis of STR Data**

**8.1 Calculating Frequencies/Probabilities Using Popstats**

- Open Popstats and click the appropriate icon to enter either a Single Sample Target Profile or a Forensic Mixture Target Profile. The selection may also be made under the Case Type pull-down menu.

**Note:** Do not maximize the window

- In the Specimen ID field, enter the lab #\_Item#
- In the Comments field, add any additional info (i.e. sperm fraction, major profile)
- Enter the alleles deemed appropriate for statistical analysis (see the section on Data Interpretation Guidelines in the Forensic Biology Procedures Manual).

**Note:** It is not necessary to enter alleles for Ameroge or the Penta loci as population data for these loci are not contained in the database.

- Click the calculator icon or select Calculate Statistics from the Profile pull-down menu.
- Click the 1/f icon or select Inverse Summary of Probability Statistics in the Window pull-down menu.
- Click the printer icon or select Print Report from the File pull-down menu. Select Forensic\BrowardRpt.exe and print. Close the print window.

**Note:** The above steps must be performed for both the Alaska Native database and the FBI STR database

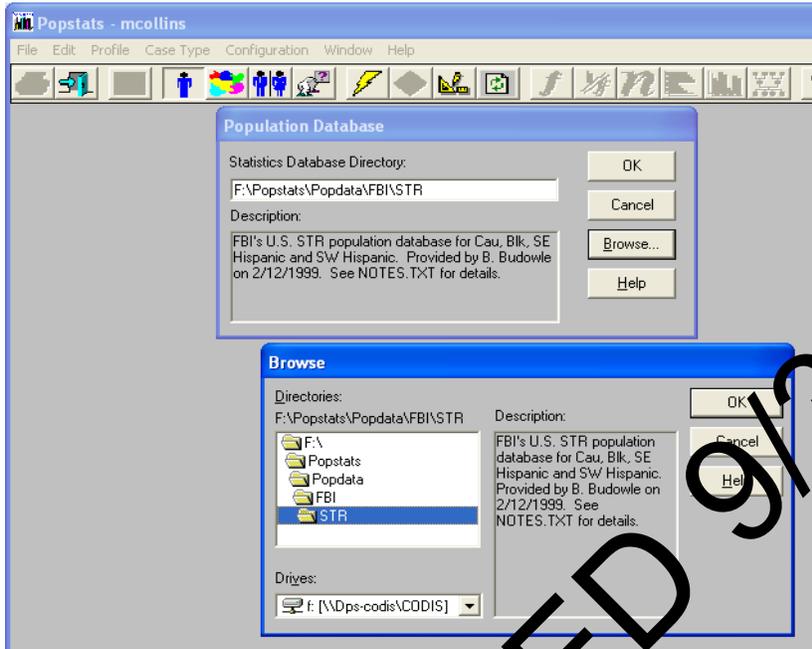
- To select a database, choose Population database from the Configuration pull-down menu.
- Select Browse in the Population Database window. Navigate to FBI/STR folder or the Alaska folder and click OK twice.

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**Selecting the Caucasian and African-American Population Databases**



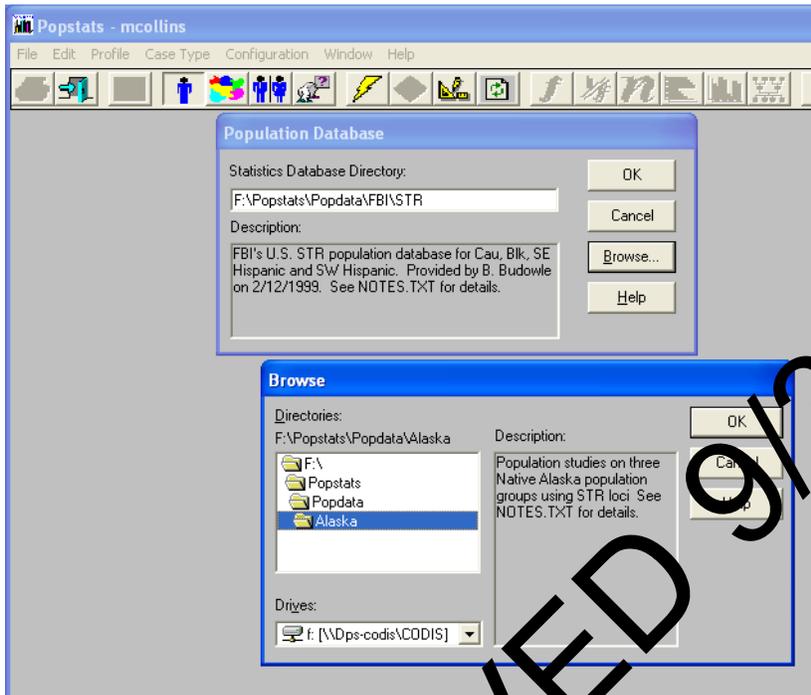
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**Selecting the Alaska Native Population Databases**



**Note:** Be sure that the correct sample type (single sample or forensic mixture) is selected after changing the population database.

- Repeat the above steps for the second population. The profile does not need to be re-entered when the database is changed but the case and item number information needs to be re-entered.

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**Section 9 Equipment / Instrument/ Lab Maintenance**

All maintenance records are maintained with the instrument. Each calendar year, records are archived in the annual Forensic Biology case record in the LIMS.

**9.1 Temperature Logs**

Temperatures for refrigerators/freezer are monitored electronically as a component of the laboratory security system. Temperatures for incubators and water baths are recorded by the analysts, when in use.

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

**9.2 Microscopes**

Reference: *BX41 System Microscope Instructions Manual*  
*Leica DM1000/Leica DM1000 LED Operating Manual*

General Instructions

- Simple dust is the number one enemy of microscopes and optical quality. When the scope is not in use, it should be covered with a plastic dust cover. Never leave a tube or an objective port open so that dust can get to the internal surfaces.
- When cleaning of the microscope stand is required, use a clean, lint free cloth lightly moistened with water containing a small amount of mild detergent. Quickly follow the cleaning by wiping with a dry lint free cloth.
- Any residue of mounting medium or immersion oil on the stand or stage should be removed immediately after examinations are completed, using a cotton-tipped swab or cloth lightly moistened with xylene. Following this solvent cleaning, the xylene should be removed as quickly as possible using a clean, dry cloth. It is wise to follow the solvent removal with the above detergent cleaning.
- Before any physical contact is made with the lens surfaces (eyepieces, objectives, condenser, field diaphragm), any loose dust or debris should be blown off using compressed gas. Any stubborn dust, dirt or oil can be removed using lens cleaning fluid and a cotton-tipped swab.

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- Proceed to clean the lens with a moistened swab by placing the tip at the center of the lens and working with light pressure toward the outside of the lens in a spiral motion. Immediately repeat this process using a dry swab. For very small objective lenses, the swab may be gently rotated between the thumb and forefinger while it contacts the lens. Examine the surface of the lens in reflected light for any evidence of smearing; if the surface is not completely clean repeat the process. When clean, a coated lens will have a uniform bluish color. It may be necessary to use a small amount of xylene to remove similar or other mounting mediums (see above).
- Scopes should be cleaned, lubricated and aligned when necessary by a competent microscope mechanic.
- If artifacts caused by dirt are seen in the microscope image, one can locate their source in the following manner:
  - If the trouble can be eliminated by a slight adjustment of the condenser, look for the cause in the lamp bulb, lamp condenser, or filter in front of it.
  - If a change of focus control eliminates the artifact, look to the condenser or specimen itself.
  - If rotation of the objective lens causes the artifact to move, the soil is obviously on the objective. Similarly, if rotation of the eyepieces causes the artifact to move, the soiling is on the eyepiece.

Operation / Troubleshooting / Maintenance

See referenced manuals

**9.3 Magni Whirl® Constant Temperature Water Bath**

Reference: *Magni Whirl® Constant Temperature Water Bath Operation and Maintenance Manual*

Operation

- Turn on main switch.
- Set Microtrol switch to LOW positions (37°C).
- The unit is now in operation and will control at the desired set point. Agitator light will cycle on and off with movement of the agitator. Heater pilot light will remain on until set point temperature is reached. At that point, the heater pilot light will cycle on and off with the heaters as control calls for heat to maintain set point temperature.

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- Allow the temperature to stabilize for 30 minutes after adjustment.

Cleaning And Maintenance

- See referenced manual.

**9.4 CORNING 430 pH meter and CORNING Electrode 476306 Deep Vessel Combination**

Operating Instructions

- Prior to use, unplug the filling hole cover at the cable connector end of the electrode.
- If necessary, add saturated KCL with a transfer pipette until the level is within one inch of the fill hole.
- Inspect the ceramic junction for any air bubbles. Remove any air bubbles by gently tapping the electrode until the bubbles are expelled.
- Follow the instructions in the image below.
- For best results, rinse the electrode with deionized water between measurements. The electrode and samples should be at room temperature prior to measurement.
- Record the date and calibration results in the Calibration Log.
- When calibration and sample measurements are completed, plug the fill hole cover and place the electrode back in the 7.0 buffer until the next use.

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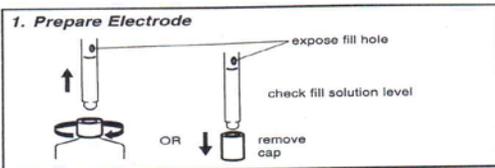
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430

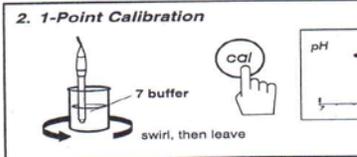
**Step-by-step guide to taking pH measurements**

**1. Prepare Electrode**



expose fill hole  
check fill solution level  
OR  
remove cap

**2. 1-Point Calibration**



7 buffer  
swirl, then leave



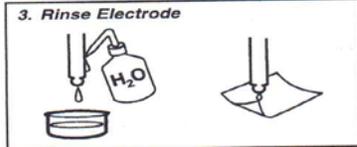
pH 7.03  
Cal 7.03

The meter will auto endpoint and display



pH 7.00  
Cal 7.00

**3. Rinse Electrode**

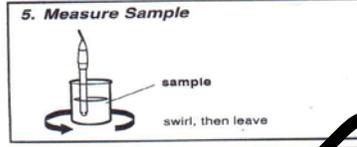


**4. 2-Point Calibration**

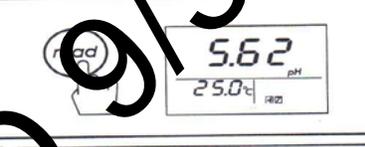


4 10  
repeat step 2 using each buffer then rinse

**5. Measure Sample**

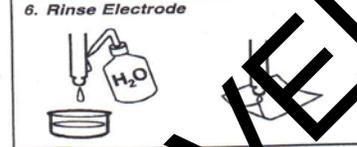


sample  
swirl, then leave

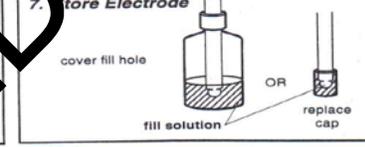


5.62  
pH  
25.0°C

**6. Rinse Electrode**



**7. Store Electrode**



cover fill hole  
fill solution  
OR  
replace cap

**CORNING**

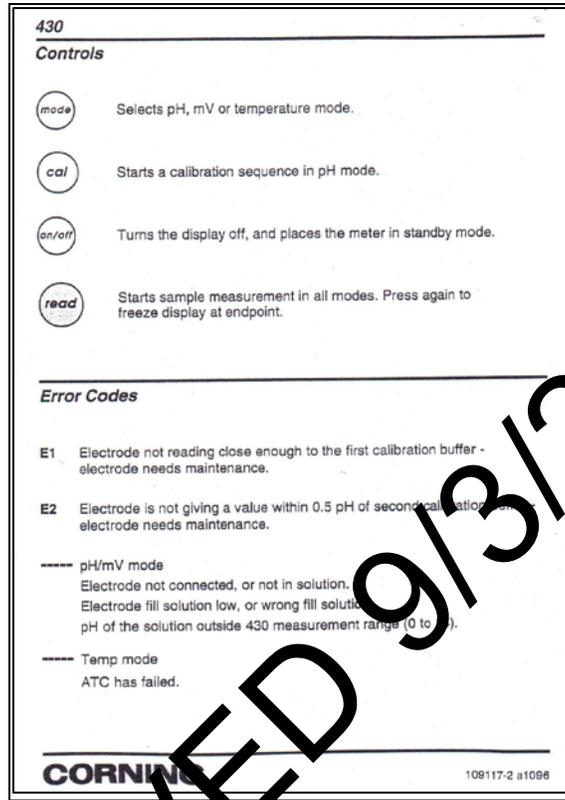
109117-2 a1096

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Maintenance and Troubleshooting

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**Precautions and Limitations**

1. **Do not** allow the electrode to run dry. Always maintain the fill solution level within one inch (2.5cm) of the fill hole.
2. **Do not** wipe the electrode tip. Blot dry with a lint-free tissue.
3. **Do not** use a KCl solution saturated with AgCl as the fill solution, as it could permanently damage the reference element.
4. **Do not** leave the electrode in organic solvents or strongly basic solutions, for extended periods. If measurements are made in these solutions, readings should be taken quickly and the electrode rinsed immediately with deionized water. After rinsing the electrode, soak it in 7.0 buffer for two hours before using.
5. **Do not** use the electrodes in solutions that exceed a temperature range of 0-100°C.
6. **Do not** use the non-replaceable junction electrodes to measure the pH of semi-solids, oils and suspended solids. These solutions are best measured with the replaceable junction electrode.
7. **Do not** use the electrode in fluoride solutions or hydrofluoric acid.

Electrode performance can be reduced as a result of prolonged use and aging of the glass membrane. If your pH electrode is exhibiting slow response, low slope values, continuous drift, or erratic readings, follow the procedures in the following image.

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**Maintenance and Troubleshooting**

Electrode performance can be reduced as a result of prolonged use and aging of the glass membrane. If your pH electrodes are exhibiting slow response, low slope values, continuous drift, or erratic readings, follow the procedures listed below.

**1. Slow or drift response**

Usually caused by a reduction in the strength of the Saturated KCl fill solution. Remove all KCl and refill with fresh.

**2. Blocked junction**

Some samples can cause the junction to become blocked. To test for this, blot the tip dry using a lint-free tissue, and let the electrode air dry for 15 minutes. If the junction is functioning properly, KCl crystals will appear on the tip of the electrode. A failure to observe crystals indicates a blocked junction.

If the junction is blocked, place the electrode in warm (50°C) deionized water for 1 hour. Repeat the preceding test and treatment until the junction flows freely. If necessary, replace the junction if you have a replaceable junction electrode. To replace the junction use the Replaceable Junction Kit (containing three junctions), Cat. No. 417289.

**3. Excess KCl crystals**

After prolonged use KCl crystals may build up inside the electrode and settle on the electrode tip, or the KCl may become discolored. Remove the old fill solution with a transfer pipet and replace it with warm deionized water to dissolve the crystals. Remove the water and fill the electrode with fresh saturated KCl solution.

**Note:** To avoid excess build up of KCl crystals, Corning recommends the fill solution be changed weekly.

**4. Cleaning the pH bulb**

a. Protein contamination: Soak the electrode bulb for 30 minutes in a 10% solution of pepsin. Rinse with deionized water and soak the electrode in 7.0 buffer for two hours before using.

b. Oil contamination: Wash the electrode with a 50% water-acetone solution. **Do not** soak the electrode in the acetone solution, as it will deteriorate the bottom seal of the plastic electrodes. Rinse with deionized water and soak the electrode in 7.0 buffer for two hours before using.

**5. Reconditioning the pH Bulb**

If the preceding maintenance and cleaning procedures fail to restore electrode performance, you need to recondition the pH bulb. Soak the electrode in a 0.1M ammonium bifluoride solution for two minutes. Rinse immediately with deionized water and soak overnight in 7.0 buffer before use.

**Warning:** Do not exceed the two minutes in the ammonium bifluoride solution. Excess time in this solution will permanently damage the electrode.

**Performance Specifications**

The Corning combination pH electrodes meet the following specifications:

pH range	0-14
Temperature Range	0-100°C
Zero Point	pH = 7.0 ± 0.5

For technical application assistance call toll-free, 1-800-222-7740.

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**9.5 Mettler PE360 Delta Range® Balance**

Records of calibration and maintenance are retained by the laboratory Quality Manager in the Quality Assurance records.

**9.6 Qiagen BioRobot EZ1 Advanced-XL**

Reference: *EZ1 Advanced XL User Manual (retained in laboratory)*  
*Qiagen supplementary protocol MA67 (Evaluating pipetting accuracy of the EZ1® Advanced XL using the EZ1 Advanced XL Test Card)*  
*Qiagen supplementary protocol MA68 (Evaluating the temperature accuracy of the EZ1® Advanced XL)*

Maintenance Procedures

Preventive Maintenance procedures are described in Section 6 of the EZ1 Advanced XL User Manual. Regular maintenance (6.1; performed after each run) and Daily maintenance (6.2; performed at the end of each day the robot is in use) procedures are performed as directed in the User Manual and do not need to be recorded.

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

**NOTE:** The instrument will give a warning when the lamp needs to be replaced. Notify the section supervisor if you receive this warning.

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

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

Performance Checks

Performance checks shall be run bi-annually, regardless of whether or not service was performed on the instrument. Additionally, any instrument having PM or service performed shall be subjected to a performance check to being used again for casework analyses. Performance checks are performed in accordance with Qiagen

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supplementary protocols MA67 and MA68 and are documented on the Maintenance Log.

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

### 9.7 *Stratagene Mx3000p/3005p*

Reference: *Mx3000P and Mx3005P QPCR Systems Setup and User's Guide*  
([http://www.genomics.agilent.com/files/Manual/Mx3000P\\_and\\_Mx3005P\\_User\\_Guide.pdf](http://www.genomics.agilent.com/files/Manual/Mx3000P_and_Mx3005P_User_Guide.pdf))

#### Maintenance Procedures

Regular maintenance is not required on the Stratagene Real Time instrument. Any preventive maintenance (PM) and service to the instrument, as well as the dates that an instrument is taken out of service or returned to service are recorded on the Mx3000P Maintenance Log (located at the end of this document).

#### Performance Checks

A performance check is performed bi-annually and recorded on the maintenance log. The Instrument Qualification Test Run (as described in the Mx3000P and Mx3005P QPCR Systems Setup and User's Guide) is used as the instrument performance check. Refer to the User's Guide for instructions on performing the test. The results from the test are retained with the maintenance records for the instrument.

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

The maintenance log form is located at the end of this document.

### 9.8 *Applied Biosystems 9700 Thermal Cyclers*

Reference: *Forensic Biology Standard Operating Procedures Manual*  
*GeneAmp® PCR System 9700 96-Well Sample Block Module User's Manual*  
([http://www3.appliedbiosystems.com/cms/groups/mcb\\_support/documents/generaldocuments/cms\\_041143.pdf](http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_041143.pdf))

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Maintenance Procedures

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

Performance Checks

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

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

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

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

**9.9 Applied Biosystems 3130xl**

*References: Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide  
Promega Protocol GE163, "PowerPlex® Matrix Standards, 3100-Custom"*

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

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### 9.9.1 As Needed Maintenance

- **Ensure adequate levels of buffer and water in reservoirs**
- Purge old plate records
  - Navigate to **GA instruments > Database Manager**
  - Click on the **Cleanup Processed Plate** in the Database Status box. This will purge the Oracle database of all of the processed plates, except spectral and spatial plate files.

#### 9.9.1.1 Replacing Buffer (1x solution prepared from Applied Biosystems 10x stock solution)

Both the anode and cathode buffer reservoirs should be changed about once a week or more often if the instrument has been running frequently. Using old buffer will result in an increase in spikes and baseline noise. All buffer and water reservoirs should be filled to the fill lines.

- Press the tray button on the outside of the instrument to bring the autosampler to the forward position and place fresh 1X genetic analyzer buffer in the cathode reservoir in position 1 (front left) as necessary.
- Place fresh de-ionized water into the reservoirs in positions 2 and 4 (back left and back right) whenever the anode and cathode buffers are replaced.
- Replace the 1X genetic analyzer buffer in the anode buffer jar on the instrument whenever the cathode buffer (position 1) is replaced.

#### 9.9.1.2 Replenishing Polymer

Polymer is replenished on an as needed basis when the bottle is almost empty or expired. Only 3130 POP-4™ is to be used.

- Allow the polymer to equilibrate to room temperature prior to placing on the instrument.
- Launch the Replenish Polymer Wizard in the Data Collection Software.  
**GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Wizards (top of screen) > Replenish Polymer Wizard**
- Follow prompts given in the wizard to load fresh polymer on the instrument.
- Polymer may be replenished as part of the Water Wash Wizard.

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**Note:** If changing the polymer or performing other maintenance, replace the 1X genetic analyzer cathode and anode buffer reservoirs after other maintenance is complete.

**9.9.1.3 Replacing the Capillary Array**

- The following indications may suggest that a new capillary array is required:
  - Poor sizing precision or allele calling
  - Poor resolution and/or decreased signal intensity
- Launch the Install Array Wizard in the Data Collection Software.  
**GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Wizards (top of screen) > Install Array Wizard**
- Follow the prompts given in the wizard to install or replace an array.

**Note:** Spatial and Spectral Calibrations must be performed anytime an array is replaced. A water wash, water trap flush, and TH01 2.3-10 Resolution must also be performed to verify performance of the array.

**9.9.1.4 Spatial Calibration**

A spatial calibration maps the pixel positions of the signal from each capillary in the spatial dimension of the CCD camera. A spatial calibration must be run when the array has been removed for cleaning or replacement.

Performing a Spatial Calibration

- Launch the Spatial Calibration  
**GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Spatial Run Scheduler**
- In the Spatial Protocols section, select one of the following:
  - If the capillaries contain fresh polymer, select Protocol > 3130SpatialNoFill\_1
  - Otherwise, select Protocol > 3130SpatialFill\_1
- Click **Start**. The calibration run lasts approximately:
  - 2 minutes without filling the capillaries
  - 6 minutes with filling the capillaries

Evaluating a Spatial Calibration

- All peaks should have similar heights
- One orange cross should mark the top of each peak.

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- The shape should be a single sharp peak for each capillary. Small shoulders are acceptable.
- Position values are 13-16 higher than the previous one for every capillary. Theoretical spacing between capillaries is 15.
- If the calibration passed click **Accept** to write the calibration data to the database.
- If the calibration failed click **Reject** then refer to the Applied Biosystems 3130/3130xl Genetic Analyzer's Maintenance, Troubleshooting, and Reference Guide.

### 9.9.1.5 Spectral Calibration

A spectral calibration creates a matrix. A spectral calibration should be performed whenever the array has been changed, the CCD camera or laser have been realigned or replaced or if you see a decrease in spectral separation.

#### Performing a Spectral Calibration

- Thaw the PowerPlex® Matrix Standards, 3100/3130. Vortex and spin briefly.
- Before mixing the dye fragments (as described below), a 1:5 dilution of each dye fragment must first be prepared by mixing 2µL of the dye fragment in 8µL of sterile de-ionized distilled water.
- A matrix standard master mix is prepared by combining the diluted dye fragments in a tube as follows:
 

○ Hi-Di™ Formamide	480µL
○ diluted FL Matrix Standard	5µL
○ diluted JOE Matrix Standard	5µL
○ diluted TMR Matrix Standard	5µL
○ diluted CXR Matrix Standard	5µL
- Vortex and spin briefly.
- On the 3130xl Genetic Analyzer, 16 wells of a 96 well plate are used for creating a matrix for the 16 capillaries. Load 25µL of the fragment mix into each of the 16 wells (i.e. A1-H2 of a 96-well plate) and cover with a plate septa.
- Briefly centrifuge the well plate to remove any bubbles.
- Denature samples at 95°C for 3 minutes then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument.
- Align the septa with the plate retainer and snap the retainer onto the reaction plate and plate base.

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- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the front of the instrument and wait for the autosampler to stop moving.
- Open the doors and place the plate assembly on the autosampler in position A or B. There is only one proper orientation for the plate, with the notched end of the plate base in the lower right corner.
- Ensure the plate assembly fits flat in the autosampler. Failure to do so may allow the capillary tips to lift the plate assembly off the autosampler.
- Once the plate is in place the position of the plate turns to yellow in the Run Scheduler view on the monitor.
- Close the instrument doors.
- Create a Plate Record  
**GA instruments > ga3130xl > Plate Manager**
- Click **New** (bottom of screen) and name the spectral with the date (i.e. SpectralYY-MMDD\_KAH).
- In the dialog box that appears, select **Spectral Calibration** in the Application drop-down list and select the **96-well plate type**. Add initials in the owner and operator windows and click **OK**.
- In the Spectral Calibration Plate Editor dialog box, enter Spectral as the sample name for each of the appropriate cells. Under the Instrument Protocol column, select the protocol **PP16\_spectral**. Ensure that this information is present for each row that contains a sample name. Click **OK**.
- Start the Run  
**GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Run Scheduler > Plate View**
- Click **Find All** screen (if necessary) and select the plate you just created. Now click on the yellow area of the plate you have just placed on the autosampler. The area should turn green once a plate is linked.
- On the top left of the screen the run button will turn green. Click on the run button. A Prompt appears stating "You are about to start processing plates" click **OK**.
- The status of the run can be monitored in the "Instrument Status" window and also in the "Capillaries Viewer" or "Cap/Array Viewer."

**Note:** Do not leave the computer on these two windows for extended periods of time due to large amounts of memory needed to view the data.

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Evaluating a Spectral Calibration

- Upon Completion of the run, check the status of the spectral run  
**GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Instrument Status**
- A minimum of 12 of the 16 capillaries should pass calibration.
- View the spectral and raw data for each capillary and verify the following:  
**GA instruments > ga3130xl > 12-F0196 (or 12-F0197) > Spectral Viewer**
  - Each capillary should have a Q-value > 0.9 and a condition number range between 3 and 12.
  - Order of the peaks in the spectral profile from left to right; blue-green-yellow-red.
  - Order of the peaks in the raw data profile from left to right; red-yellow-green-blue.
- If less than the recommended number of capillaries pass, the spectral calibration should be repeated. First, re-inject the standards to see if the minimum number of capillaries pass. If re-injecting does not work, then create a matrix standard master mix prepared by combining the 1:5 diluted dye fragments (as prepared in 9.1.6.1) in a tube as follows and repeat the spectral calibration:
 

○ Hi-Di™ Formamide	460µL
○ diluted FL Matrix Standard	10µL
○ diluted JOE Matrix Standard	10µL
○ diluted TMR Matrix Standard	10µL
○ diluted CXR Matrix Standard	10µL
- For additional troubleshooting see *PowerPlex® Matrix Standards, 3100/3130 Part #TBD022* or Applied Biosystems 3130/3130xl Genetic Analyzer's Getting Started Guide.
- If the results are acceptable, the spectral calibration is automatically active for that dye set and saved as day, date, and time of run.

**9.9.2 Monthly Maintenance**

- Run the Water Wash Wizard, flush water trap, and perform TH01 9.3-10 resolution.
- Defragment the hard drive  
**Start > All Programs > Accessories > System Tools > Disk Defragmenter**
- Wipe down 3130xl (inside and out)

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### 9.9.2.1 Water Wash, Water Trap Flushing, and TH01 9.3-10 alleles Resolution/Performance Check

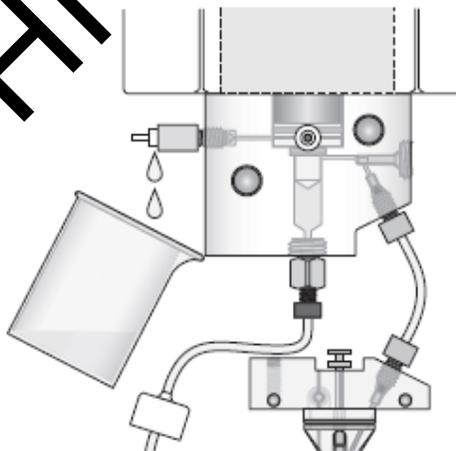
The water wash, water trap flush, and TH01 9.3-10 Resolution are performed as part of monthly maintenance and/or anytime an array is replaced. A water wash may also be performed when there is a noticeable decrease in the data quality that was not resolved by replacing the cathode and anode buffer vessels.

#### Performing the Water Wash

- Launch the Water Wash Wizard  
**GA Instruments > ga3130xl > 12-F0196 (or 12-F0197) > Wizards (top of screen) > Water Wash Wizard**
- Follow the wizard instructions to completion.

**Exception:** A 15mL conical (filled to about 10mL) is used in place of the plastic bottle to hold the water for the wash. The plastic tube is inserted to the bottom of the conical to prevent aspiration of air into the pump block.

#### Flushing and Filling the Water Trap



- Flush the water trap each time you perform the Water Wash Wizard.
- Fill a 20ml syringe with **WARM** distilled or deionized H<sub>2</sub>O. Expel any bubbles from the syringe.

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- Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe onto the fitting clockwise with the other hand.
- Open the Luer fitting by grasping the body of the fitting and turning it and the attached syringe approximately one-half turn counterclockwise.
- Open the exit fitting at the top left side of the pump block by turning it approximately one-half turn counterclockwise.
- Hold empty beaker under the exit fitting. Flush the trap by steadily pushing approximately 5ml of H<sub>2</sub>O through the syringe plunger (**DO NOT USE EXCESSIVE FORCE**).
- Close the Luer fitting by turning one-half turn clockwise.
- Close the exit fitting by turning one-half clockwise
- Remove the syringe from the Luer fitting while holding the fitting with one hand while turning the syringe counterclockwise with the other hand.

### TH01 9.3-10 alleles Resolution/Performance Check

A performance check allows you to periodically self-check the instrument system's resolution and its ability to correctly size peaks within one base pair using Promega PowerPlex® 16 Classic Allelic Ladder.

Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:

- 20µl ILS 600
- 20µl allelic ladder
- 180µl Hi-Di™ Formamide
- Vortex the master mix and spin briefly. Transfer 11µl of the master mix to the appropriate wells (i.e. A1-H2).
- Place septa onto the 96-well plate.
- Briefly centrifuge the well plate to remove any bubbles. Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button to bring the autosampler to the forward position and place the plate in the autosampler (position A or B) with placement of the notched corner in the lower right.
- Close the instrument doors.
- Creating a Plate Record. (Note: All sample names can be designated by AL)
- Linking the Plate and Starting the Run.

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- Electropherograms zoomed into the 9.3-10 TH01 locus are printed and retained in the instrument binder.

The above test checks the resolution of the system.

The allelic ladders will be run to perform the TH01 locus 9.3 and 10 alleles single bp resolution check. The peaks will be deemed clearly defined if (i) both these peaks are above the analytical (reporting) threshold of 100RFU and (ii) both these allelic peaks are correctly called and labeled.

- Injection “positive” control: Inspection of all the loci and all the allelic peaks at all the loci in the allelic ladder will be conducted to ensure that all standard peaks (Promega PowerPlex16 manual) are above the analytical threshold and correctly called and labeled.

- Injection “negative” control: A mixture consisting of only Formamide and PowerPlex16 ILS in appropriate proportions (listed earlier) will be injected to ensure that (i) the ILS peaks are properly defined and labeled and are above the analytical/reporting threshold and (ii) no artifacts or spurious “allelic” peaks are observed above the analytical/reporting threshold.

(Alternatively or in addition to I, a negative amplification control may also be run to perform the above test – but the first option is preferable since it checks the performance of the genetic analyzer components without introducing artifacts that may be PCR amplification related and not necessarily reflect a problem with the CE process).

Failure to achieve the above expected results will require a repeat run and/or performance check and/or additional tests until the system demonstrates expected results.

### 9.10 Applied Biosystems 3500xl

References: Applied Biosystems 3500xl Genetic Analyzers Reference Guide

PowerPlex®16 System part #TBD022, revised 8/10

PowerPlex® Matrix Standards, 3100/3130 Part #TBD022 Revised 12/10

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

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**9.10.1 Maintenance to be performed as needed**

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

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

**9.10.1.1 Replacing Anode Buffer Container (ABC)**

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

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

**9.10.1.2 Replacing Cathode Buffer Container (CBC)**

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

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

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### 9.10.1.3 Replenishing Polymer

Applied Biosystems recommends replacing the polymer (POP4) after 7 days however; the software allows you to continue using past 7 days. The polymer must be replaced after 960 samples or 120 injections.

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

### 9.10.1.4 Replacing the Capillary Array

Applied Biosystems recommends replacing the capillary array after 160 injections however; the software allows you to continue using past 160 injections.

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

**Note:** Spatial and Spectral Calibrations must be performed anytime an array is replaced. A water wash, water trap flush and TH01 9.3-10 Resolution must also be performed to verify performance of the array.

### 9.10.1.5 Spatial Calibration

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

#### Performing a Spatial Calibration

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

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Evaluating a Spatial Calibration

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

**9.10.1.6 Spectral Calibration**

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

Performing a Spectral Calibration

- In the Dashboard, Click **Start Pre-heat** at least 30 minutes prior to the start of the run.
- Ensure the consumables are not expired and adequate injections remain.
- Ensure the pump assembly is free of bubbles, run the Remove bubble wizard if needed.
- Thaw the PowerFlex® Matrix Standards. Vortex and spin briefly.
- Make a 1:10 dilution of each dye fragment by mixing 2µl of the dye fragment in 18µl of sterile de-ionized distilled water.
- A matrix standard master mix is prepared by combining the diluted dye fragments in a tube as follows:
 

○ Hi-Di™ Formamide	668µl	
○ diluted FL Matrix Standard	8µl	
○ diluted JOE Matrix Standard		8µl
○ diluted TMR Matrix Standard		8µl
○ diluted CXR Matrix Standard		8µl
- Vortex and spin briefly.

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- On the 3500xl Genetic Analyzer, 24 wells of a 96 well plate are used for creating a matrix for the 24 capillaries. Load 25 µl of the matrix standard master mix into each of the 24 wells and cover with a plate septa.

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

- Briefly centrifuge the plate containing the standards and verify that each sample does not contain bubbles and is positioned correctly in the bottom of the well.
- Denature samples at 95°C for 3 minutes then snap chill for 3 minutes.
- Place the sample plate into the plate base provided with the instrument.
- Snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Access the Spectral Calibration screen:
  - Select **Maintenance**, then click **Spectral Calibration** in the navigation pane.
- Select **96** for the number of wells in the spectral calibration plate and specify the plate location (A or B) in the instrument.
- Select **Matrix Standard** as the chemistry standard and **Promega4dye** as the dye set.
- Select **Allow Borrowing**.
- Click **Start Run**.

### Evaluating a Spectral Calibration

- Passing and failing capillaries are shown in green and red respectively. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. Up to three adjacent-capillary borrowing events are allowed.
- If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times.
- View the spectral and raw data for each capillary. Ensure that the data meet the following criteria:

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- Order of the peaks in the spectral profile from left to right blue-green-yellow-red.
- Order of the peaks in the raw data profile from left to right red-yellow-green-blue
- The Quality Value is  $\geq 0.95$  and the Condition Number is  $\leq 8.5$
- If the data for all capillaries meet the above criteria, click **Accept Results**.
- If any capillary data does not meet the criteria click **Reject Results** and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User guide "Spectral calibration troubleshooting" page 301.
- If the results are acceptable, click **Export Spectral Calibration Results**. Click **View Spectral Calibration Report**, click **Print**, select **Super DF Writer**, specify a name (i.e. Spectral Report 3500xl 03-03-2011 SEJ) for the report and save the file under DNA\_Share in the 3500xl equipment maintenance folder.

### 9.10.2 Monthly Maintenance

- Run the Water Wash Wizard, flush water trap, and perform TH01 9.3-10 Resolution
- Defragment the hard drive  
**Start > Programs > Accessories > System Tools > Disk Defragmenter**
- Check for computer updates  
Start > type in **Windows Update** in the search bar. Click on **Windows Update**
- Wipe down the 3500xl (inside and out)

#### 9.10.2.1 Water Wash, Water Trap Flushing, and TH01 9.3-10 alleles Resolution/Performance Check

The water wash, water trap flush, and TH01 9.3-10 resolution are performed as part of monthly maintenance and/or anytime an array is replaced.

##### Performing the Water Wash

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

**Note:** An empty ABC reservoir may be used instead of emptying the reservoir currently on the instrument. Simply remove from the instrument, cover, and set aside. At the

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completion of the Water Wash Wizard replace the ABC with the reservoir previously removed from the instrument or a new reservoir.

Flushing the Water Trap

Perform this to prolong the life of the pump and to clean any diluted polymer.

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

TH01 9.3-10 alleles Resolution/Performance Check

A performance check allows you to periodically self-check the instrument system's resolution and its ability to correctly size peaks within one base pair using Promega PowerPlex® 16 Classic Allelic Ladder. A 9.3-10 Resolution should be performed whenever the capillary array has been changed and as part of the monthly maintenance of the instrument.

- Prepare a loading master mix by adding the following volumes of reagents to an appropriately sized tube:
  - 11µl ILS 600
  - 20µl allelic ladder
  - 285µl Hi-Di Formamide
- Vortex the master mix and spin briefly. Transfer 11µl of the master mix to the appropriate wells (i.e. A1-H3).
- Briefly centrifuge the well plate to remove any bubbles. Denature samples at 95°C for 3 minutes and then snap chill for 3 minutes.
- Place the 96-well plate into the plate base provided with the instrument and snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.

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- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler.
- Close the instrument doors.
- Creating a Plate Record (Note: Sample names can be designated with AL)
- Linking the Plate and Starting the Run

The above test checks the resolution of the system.

The allelic ladders will be run to perform the TH01 locus 9.3 and 10 alleles single bp resolution check. The peaks will be deemed clearly defined if (i) both these peaks are above the analytical (reporting) threshold of 100RFU and (ii) both these allelic peaks are correctly called and labeled.

- Injection “positive” control: Inspection of all the loci and all the allelic peaks at all the loci in the allelic ladder will be conducted to ensure that all standard peaks (Promega PowerPlex16 manual) are above the analytical threshold and correctly called and labeled.

- Injection “negative” control: A mixture consisting of only Formamide and PowerPlex16 ILS in appropriate proportions (listed earlier) will be injected to ensure that (i) the ILS peaks are properly defined and labeled and are above the analytical/reporting threshold and (ii) no artifacts or spurious “allelic” peaks are observed above the analytical/reporting threshold.

(Alternatively or in addition to, a negative amplification control may also be run to perform the above test – but the first option is preferable since it checks the performance of the genetic analyzer components without introducing artifacts that may be PCR amplification related and not necessarily reflect a problem with the CE process).

Failure to achieve the above expected results will require a repeat run and/or performance check and/or additional tests until the system demonstrates expected results.

### 9.11 DNA Pipettes

DNA pipettes are tracked on an uncontrolled spreadsheet and in the LIMS (DNA case record) as items of evidence. The spreadsheet contains the location, serial number, date of last calibration, and calibration due date for each pipette) with the date of calibration and due date for the next calibration. This spreadsheet is updated each time

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pipettes are sent to the vendor (Rainin) for calibration. ISO certificates of calibration received from the vendor are archived in the LIMS.

### 9.12 *Thermometers*

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

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DNA Critical Reagent Verification Form

Analyst:

Date:

Lot #

Expiration Date

EZ1 Kits

EZ1 Reagent Cartridges  
Proteinase K  
G2 Buffer  
Carrier RNA

DTT

Quantifiler Kit

Standard  
Primer  
Rxn Mix

PowerPlex 16 Kit

Gold Star Buffer  
Primer Pair  
9947A Positive  
Allelic Ladder  
ILS 600

Amplitag Gold

Sterile Water

Ethanol (absolute, 200proof)

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**Equipment and Instrument Inventory**

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

If the instrument/equipment is removed from service permanently, the equipment/instrument inventory will be updated to reflect this action when the next revision to this manual occurs.

<u>Location</u>	<u>Instrument/Equipment</u>	<u>Serial Number</u>	<u>State of Alaska Tag # or other Identifier</u>
Casework Ext. Lab 1	Computer	10146396	
Casework Ext. Lab 1	Computer	10146394	
Casework Ext. Lab 1	Computer	10146389	
Casework Ext. Lab 1	Computer	10151014	
Casework Ext. Lab 1	Eppendorf Centrifuge- 5417C		12-39089
Casework Ext. Lab 1	Barnstead/Thermolyne vortex	1329050248181	Model # M16715
Casework Ext. Lab 1	Barnstead/Thermolyne vortex	268010225853	Model # M16715
Casework Ext. Lab 1	Barnstead/Thermolyne vortex	1329040266118	Model # M16715
Casework Ext. Lab 1	Barnstead/Thermolyne vortex	1329040265980	Model # M16715
Casework Ext. Lab 1	Vortex- Sybron/Thermolyne	268	Model # M-16715
Casework Ext. Lab 1	National Labnet Mini Centrifuge		Model # C-1200
Casework Ext. Lab 1	Sorvall- MC 12V (centrifuge)		12-28851
Casework Ext. Lab 1	Barnstead- Diamond NANOpure system		12-F0518
Casework Ext. Lab 1	VWR- Mini Vortexer	29016	
Casework Ext. Lab 1	VWR Galaxy Mini Centrifuge	4030086	
Casework Ext. Lab 1	VWR Galaxy Mini Centrifuge	4030013	
Casework Ext. Lab 1	Qiagen Biorobot EZ 1 XL-1	L094A0052	12-F0190
Casework Ext. Lab 1	Qiagen Biorobot EZ 1 XL-2	L094A0050	12-F0191
Casework Ext. Lab 1	Beckman Coulter- Microfuge 18 Centrifuge	MFA01K047	
Casework Ext. Lab 1	C.B.S. Scientific (PCR Hood)		Model # P-030-202
Casework Ext. Lab 1	Barnstead/Lab-Line Model 120 (Incubator # 1)	1460061100527	
Casework Ext. Lab 1	Barnstead/Lab-Line Model 120 (Incubator # 2)	1460060537403	
Casework Ext. Lab 1	Sanyo Model SR-4433S (Refrigerator # 1)	S/N: 080213357	
Casework Ext. Lab 1	Sanyo Model SR-4433S (Refrigerator # 2)	S/N: 060526580	
Casework Ext. Lab 1	GE Model (Refrigerator/Freezer #1)		CASEWORK EXT LAB #1 REFRIGERATOR/FREEZER #1
Casework Ext. Lab 1	Woods Model (Freezer #1)		CASEWORK EXT LAB #1 FREEZER #1

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<u>Location</u>	<u>Instrument/Equipment</u>	<u>Serial Number</u>	<u>State of Alaska Tag # or other Identifier</u>
Casework Ext. Lab 2	Computer		10151016
Casework Ext. Lab 2	Computer		10004364
Casework Ext. Lab 2	Qiagen Biorobot EZ 1 XL-3		12-F0194
Casework Ext. Lab 2	Qiagen Biorobot EZ 1 XL-4		12-F0574
Casework Ext. Lab 2	Eppendorf Centrifuge 5424	5424Y1723924	
Casework Ext. Lab 2	Eppendorf Centrifuge 5424	5424Y1423926	
Casework Ext. Lab 2	Spectrafuge Mini	0210 1288	
Casework Ext. Lab 2	VWR Galaxy Mini Star	0911 0010	
Casework Ext. Lab 2	VWR Galaxy Mini Star	0911 0011	
Casework Ext. Lab 2	ThermoScientific Vortex Maxi Mixi	1858001027119	
Casework Ext. Lab 2	ThermoScientific Vortex Maxi Mixi	01858100929500	
Casework Ext. Lab 2	C.B.S. Scientific (PCR Hood)	S/N: 302176	
Casework Ext. Lab 2	ThermoScientific 120 (Incubator #1)	146009133691	
Casework Ext. Lab 2	ThermoScientific 120 (Incubator #2)	1460100261435	
Casework Ext. Lab 2	Marvel Model 4570101 (Refrigerator)	20647	
Casework Ext. Lab 2	Kenmore Model (Refrigerator/Freezer)	BA02732592	

<u>Location</u>	<u>Instrument/Equipment</u>	<u>Serial Number</u>	<u>State of Alaska Tag # or other Identifier</u>
Database Extraction Lab	Computer (connected to M48)	HUB61803RT	
Database Extraction Lab	Computer (on desk)	10163406	
Database Extraction Lab	Photocopier (connected to Qiagility)		12-F0578
Database Extraction Lab	M48-1	60210297	
Database Extraction Lab	Qiagility		12-F0575
Database Extraction Lab	Barnstead/Thermolyne vortex	1329070234047	Model # M16715
Database Extraction Lab	Barnstead/Thermolyne vortex	1329070234013	Model # M16715
Database Extraction Lab	VWR Galaxy Mini Centrifuge	0709 0321	
Database Extraction Lab	VWR Galaxy Mini Centrifuge	0803 0699	
Database Extraction Lab	Eppendorf Centrifuge 5424	5424 #0013369	
Database Extraction Lab	C.B.S. Scientific (PCR Hood)		Model # P-030-202
Database Extraction Lab	Barnstead/Lab-Line Model 120 (Incubator #1)	1460071203421	
Database Extraction Lab	Compact Freezer Model CMF1551L	S/N: 0710410027	
Database Extraction Lab	GE Model SC4DLC	TF139382	

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<u>Location</u>	<u>Instrument/Equipment</u>	<u>Serial Number</u>	<u>State of Alaska Tag # or other Identifier</u>
DNA Post-PCR Lab	Computer (connected to 3130-1)		12-F0197
DNA Post-PCR Lab	Computer (connected to 3130-2)		12-F0196
DNA Post-PCR Lab	Computer (connected to 3500)		10173649
DNA Post-PCR Lab	Computer (connected to Stratagene)		12-F0200
DNA Post-PCR Lab	9700-2 (Thermocycler) Base	805S4062812	12-39116
DNA Post-PCR Lab	9700-2 (Thermocycler) Block	096S4060309	12-39116
DNA Post-PCR Lab	9700-4 (Thermocycler) Base	805S701136	10140660
DNA Post-PCR Lab	9700-4 (Thermocycler) Block	096S704273	10140660
DNA Post-PCR Lab	9700-5 (Thermocycler) Base	805S701030	10140661
DNA Post-PCR Lab	9700-5 (Thermocycler) Block	096S7042308	10140661
DNA Post-PCR Lab	9700-6 (Thermocycler) Base	805S701183	10140663
DNA Post-PCR Lab	9700-6 (Thermocycler) Block	096S7102713	10140663
DNA Post-PCR Lab	9700-7 (Thermocycler) Base	805S7100137	10140664
DNA Post-PCR Lab	9700-7 (Thermocycler) Block	096S7102739	10140664
DNA Post-PCR Lab	Heraeus Instruments L centrifuge 40		12-39113
DNA Post-PCR Lab	Stratagene Mx3000	S/N: 0407437	12-39114
DNA Post-PCR Lab	Applied Biosystems/Hitachi- 3500XL	22308-150	
DNA Post-PCR Lab	Applied Biosystems/Hitachi- 3100-1	1602-018	
DNA Post-PCR Lab	Applied Biosystems/Hitachi- 3100-2	17210-015	
DNA Post-PCR Lab	Tomy Capsule Model # HF-120	709311	
DNA Post-PCR Lab	Barnstead/Thermolyne vortex	268	Model # M16715
DNA Post-PCR Lab	National Labnet Mini Centrifuge		Model # C-1200
DNA Post-PCR Lab	GE Model GMR04HANBBS	TF042419	POST-PCR LAB REFRIGERATOR #1
DNA Post-PCR Lab	Kenmore Model (Refrigerator #2)		POST-PCR LAB REFRIGERATOR #2
DNA Post-PCR Lab	Caravell Model (Freezer #1)		POST-PCR LAB FREEZER #1
DNA Post-PCR Lab	Alpha Technics Thermometer	101402624	
DNA Post-PCR Lab	Alpha Technics Temperature Probe	512898	
DNA Post-PCR Lab	Alpha Technics Thermometer	02E400763	
DNA Post-PCR Lab	Alpha Technics Temperature Probe	510675	
DNA Post-PCR Lab	VWR Standard Heatblock	S/N: 050408002	

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<u>Location</u>	<u>Instrument/Equipment</u>	<u>Serial Number</u>	<u>State of Alaska Tag # or other Identifier</u>
Screening Area (3 rooms)	Computer		10153508
Screening Area (3 rooms)	Computer		0160175
Screening Area (3 rooms)	Mettler PE360 (digital scale)	E49599	
Screening Area (3 rooms)	Corning pH meter model 430	S/N: 001112	
Screening Area (3 rooms)	Corning hot plate stirrer		Model PC-351
Screening Area (3 rooms)	Wild Heerbrugg stereoscope	F08703	12-31720
Screening Area (3 rooms)	Wild Heerbrugg stereoscope		12-27938
Screening Area (3 rooms)	Fumehood-4	101104	
Screening Area (3 rooms)	Leica MZ16 microscope		12-F0504
Screening Area (3 rooms)	Leica MZ16 microscope		12-F0503
Screening Area (3 rooms)	Boyle Instruments Olympus microscope	12-27936	
Screening Area (3 rooms)	Barnstead/Thermolyne vortex	1329050886614	Model # M16715
Screening Area (3 rooms)	VWR Scientific incubator model 1500E	S/N: 0301994	12-28832
Screening Area (3 rooms)	Omniprint 1000B-110 ALS (Alternative Light Source)	1087	
Screening Area (3 rooms)	Omniprint 1000B-110 ALS (Alternative Light Source)		12-F0508
Screening Area (3 rooms)	Omniprint 1000B-110 ALS (Alternative Light Source)		12-F0498
Screening Area (3 rooms)	Leica CM 1000LE microscope	324498-082010	
Screening Area (3 rooms)	MagniWhisk Constant Temperature Bath	M5-11103	
Screening Area (3 rooms)	GE C3SSAWG refrigerator	TH112260	
Screening Area (3 rooms)	VWR Galaxy Mini Centrifuge	S/N: 04010123	
Screening Area (3 rooms)	Brandford pipette	10379500	
Screening Area (3 rooms)	Rainin P10 pipette	J0501253G	
Screening Area (3 rooms)	Gilson pipette		Pipetman P10
Screening Area (3 rooms)	Gilson pipette		Pipetman P200
Screening Area (3 rooms)	Olympus BX 41 (comparison microscope) optical bridge	S/N: 030805	
Screening Area (3 rooms)	Olympus BX 41 (comparison microscope) left scope	5A19713	
Screening Area (3 rooms)	Olympus BX 41 (comparison microscope) right scope	4L20232	
Screening Area (3 rooms)	Labconco vacuum pump		12-F0573
Screening Area (3 rooms)	NCL-150 microscope light sources	4020096	
Screening Area (3 rooms)	NCL-150 microscope light sources		12-37206











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**EZ1 Advanced-XL Maintenance Log for Calendar Year \_\_\_\_\_**  
**Alaska State Tag # \_\_\_\_\_**  
**S/N: \_\_\_\_\_**

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

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**EZ1 ADVANCED-XL PIPETTING ACCURACY TEST**

Alaska State Tag # \_\_\_\_\_ S/N \_\_\_\_\_

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

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

**Pipetting 100µL of water**

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

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Pipetting 500 $\mu$ L of water

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

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**EZ1 ADVANCED-XL LEAKAGE TEST**

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

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

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**EZ1 ADVANCED-XL TEMPERATURE ACCURACY TEST**

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

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

**Equipment used**

**Laboratory Balance**

Make/Model: \_\_\_\_\_

Serial Number: \_\_\_\_\_

Last Calibration Date: \_\_\_\_\_

**Thermometer**

Make/Model: \_\_\_\_\_

Serial Number: \_\_\_\_\_

Last Calibration Date: \_\_\_\_\_

**Analyst/Technician:** \_\_\_\_\_

**Date:** \_\_\_\_\_

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StratageneMX3000/3005p Maintenance Log for Calendar Year \_\_\_\_\_  
 Alaska State Tag # \_\_\_\_\_  
 S/N: \_\_\_\_\_

Date	Task Completed (analyst initial in box)	Comments
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	
	<input type="checkbox"/> Mx3000P performance check completed <input type="checkbox"/> service call <input type="checkbox"/> in-house <input type="checkbox"/> send out	

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9700-\_\_ Diagnostics and Performance Tests

Alaska State Tag # \_\_\_\_\_  
Base S/N \_\_\_\_\_  
Block S/N \_\_\_\_\_

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

Date: \_\_\_\_\_

Operator: \_\_\_\_\_

Liquid Crystal Display (Disp)

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

Keypad Diagnostic (Keypad) Pass or Fail

Cool and Heat Rate Test

Heating Rate: \_\_\_\_\_ °C/sec Pass or Fail  
Cooling Rate: \_\_\_\_\_ °C/sec Pass or Fail

Cycle Performance Test

Average Cycle Time: \_\_\_\_\_ sec Pass or Fail

Cycle time STD: \_\_\_\_\_ sec Pass or Fail

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**9700-\_\_ Temperature Non-Uniformity Test**

Alaska State Tag # \_\_\_\_\_  
Base S/N \_\_\_\_\_  
Block S/N \_\_\_\_\_

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

Date		
Tested By		
Probe Serial No.		
Thermometer Serial No.		
Setpoint Value	94 °C	37 °C
A1		
A12		
C4		
C8		
F		
F9		
H1		
H12		

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**Temperature Non-Uniformity**

**Test Results (Pass/Fail)**

94°C \_\_\_\_\_  
37°C \_\_\_\_\_

\_\_\_\_\_  
\_\_\_\_\_





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**Appendix A Revision History**

FBWI2012 R0 Page	FBWI2011 R0 page	Location	Revision made
		Entire document	Changed "Issued" to "Approved" in footer
1	1	Document Structure	<b>Added</b> note to indicate section of manual approved by Biological Screening Technical Lead.
3	3	Section 1	<p><b>Revised: Verification</b></p> <p>Buffer G2 (when purchased outside of a kit) Purchased from Qiagen and stored at room temperature; expires on the date specified by vendor or 10 years from date received if expiration date is not specified by the vendor.</p> <p><u>Verification</u> Extract and amplify a reference sample (a sample whose genetic profile was previously established by the laboratory e.g. a previously typed convicted offender sample / known casework sample / buccal swab from a laboratory employee). A corresponding reagent blank will also be processed with the new kit lot. Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold)) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the</p>

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			<p>corresponding reagent blank. The reagent will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.</p>
3-4	3	Section 1	<p><b>Revised: Verification</b></p> <p><b>Buffer MTL</b>                  Purchased from Qiagen and stored at room temperature; expires on the date specified by vendor or 10 years from date received if expiration date is not specified by the vendor.</p> <p><u>Verification</u>                  Extract and amplify a reference sample (a sample whose genetic profile was previously established by the laboratory e.g. a previously typed convicted offender sample / known casework sample / buccal swab from a laboratory employee). A corresponding reagent blank will also be processed with the new kit lot.</p> <p>Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding reagent blank.. The reagent will be retested if the above criteria are not met and if the</p>

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			lot fails the verification twice, the lot will not be used in forensic DNA analysis.
<p><b>4</b></p>	<p><b>3</b></p>	<p><b>Section 1</b></p>	<p><b>Revised: Verification</b></p> <p><b>DTT (1M)</b></p> <p><u>Working Solution</u>                  Dissolve 0.77g dithiothreitol in 5mL sterile de-ionized water in a sterile conical tube. Add 50µL of 3M Sodium Acetate buffer solution, pH 5.2. Do not autoclave. Aliquot (0.1mL recommended) and store at -20°C. Aliquots expire one year from date of first thaw.</p> <p><u>Verification</u>                  Extract and amplify a reference semen sample (a sample whose genetic profile was previously established by the laboratory). A corresponding reagent blank with the new DTT lot will also be processed with the reference semen sample.                  Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding reagent blank.                  The reagent will be retested if the above criteria are not met and if the lot fails the verification twice, the lot</p>

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			will not be used in forensic DNA analysis.
4	4	Section 1	<p><b>Revised: Verification</b></p> <p><b>Ethanol (Absolute, 200 proof)</b></p> <p><u>Verification</u>                  Extract and amplify a reference sample and a corresponding reagent blank with the new Ethanol lot.                  Please see procedure and performance criteria for <b>Sterile De-ionized Water (H2O)</b></p>
5	4	Section 1	<p><b>Revised: Verification</b>                  EZ1 Investigator Kit</p> <p>Extract and amplify a reference sample (a sample whose genetic profile was previously established by the laboratory e.g. a previously typed convicted offender sample / known casework sample / buccal swab from a laboratory employee). A corresponding reagent blank will also be processed with the new kit lot.</p> <p>Performance criteria for acceptance of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold)) is obtained from the reference sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding reagent blank..</p>

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			<p>The reagent will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.</p>
8-9	8	Section 1	<p><b>Revised: Verification</b></p> <p><b>PowerPlex® 16 Amplification and Typing Kit</b></p> <p>Components: 9947A DNA, 10X Primer Pair mix, Gold ST*R 10X Buffer, Allelic Ladder, Internal Lane Standard (LS 500)</p> <p>Purchased from Promega Corporation. 9947A is diluted, aliquotted and stored at -20°C. Other components are stored according to manufacturer's instructions. 9947A expires one year from date of dilution. Other components expire on kit expiration date provided by manufacturer.</p> <p>Use 975µL sterile de-ionized H2O to bring the 9947A to an appropriate concentration for setting up amplification reactions.</p> <p>Amplify the 9947A positive control or an Internal Control Standard, a negative water control and a known sample twice using (1) the primers and buffer from the kit currently in use and the (2) the primers and buffer from the kit being verified, to compare amplification sensitivity and overall efficiency of amplification.</p> <p>Performance criteria for acceptance</p>

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			<p>of reagent: A full interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold)) is obtained from the positive control and no spurious peaks or artifacts are observed above the analytical/reporting threshold for the corresponding negative water control blank.</p> <p>The kit will be retested if the above criteria are not met and if the lot fails the verification twice, the lot will not be used in forensic DNA analysis.</p>
9	8	Section 1	<p><b>Revised: Verification</b></p> <p><b>Proteinase K Solution</b>                  (when purchased outside of a kit)                  Purchased from Qiagen or another suitable vendor and stored at room temperature; expires on date provided by manufacturer or 10 years from date received if no expiration date is given.</p> <p><u>Verification</u>                  Extract and amplify a reference sample and a corresponding reagent blank with the new Proteinase K lot.                  Please refer to acceptance criteria for Taq Polymerase.</p>
9-10	9	Section 1	<p><b>Revised: Verification:</b></p> <p><b>Quantifiler (QF) Kit</b></p> <p><u>Components:</u> DNA Standard A, Human Primer Mix and Reaction Mix</p>

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			<p>Purchased from Applied Biosystems. DNA Standard A and Primer Mix are stored at -20°C. Reaction Mix is stored at 2-8°C. Components expire on date provided by manufacturer. Standard curve prepared fresh approximately weekly.</p> <p><u>Verification</u>                  Test new Q<sub>1</sub> lot with a standard curve and NTCs. Expected results per manufacturer's information and specifications and /or comparison to previous lot of QF. Retest if the above criteria are not met with first testing and reject kit if the test lot fails after three verifications(prepare fresh standard curve after failure to check if problem was with the manner in which the reagent was prepared).</p>
<p>10-11</p>	<p>10</p>	<p>Section 1</p>	<p><b>Revised: Verification</b></p> <p>Sterile De-ionized Water (H<sub>2</sub>O)</p> <p>Fill glass bottles with nanopure de-ionized H<sub>2</sub>O. Autoclave for 30 minutes and store at room temperature. Expires 1 year from date prepared.</p> <p>When autoclaving, include a Sterikon™ plus Bio-indicator, or equivalent. The DNA Technical Manager must approve use of reagents autoclaved without a Sterikon™ (or equivalent). This approval will be documented in the</p>

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			<p>Reagent Log.</p> <p>After autoclaving, place the autoclaved ampoule, and an ampoule that was not autoclaved, in the 56°C incubator for 48 hours. Refer to the manufacturer's instructions to evaluate the results of the ampoules.</p> <p><u>Verification</u>                  Amplify the 99-7A control or similar acceptable positive control sample along with a negative amplification control using the new water lot. Performance criteria for acceptanc : A fully interpretable genetic profile (a minimum of all 13 FBI core loci with all alleles above the analytical/reporting threshold)) is obtained from the positive control sample and no spurious peaks or artifacts are observed above the analytical/reporting threshold.</p>
-	5	<b>Section 1</b>	<b>Removed</b> MagAttract DNA Mini M48 Kit from section 1
11	11	<b>Section 2.1</b>	<b>Revised procedure</b> to reflect that the performance check of the Alternate Light Source will be documented in the bench notes.
-	21	<b>Section 3.1</b>	<b>Removed</b> Section 3.1 and re-numbered remaining subsections of Section 3 pertaining to Qiagen BioRobot M48.
24	24	<b>Section 3.1.2 (formerly 3.2.2)</b>	<p>"Incubate in a 56°C incubator."</p> <p><b>Changed to</b></p>

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			"Incubate at 56°C."
26	26	<b>Section 3.1.3 (formerly 3.2.2)</b>	"Incubate overnight in a 37°C incubator."  <b>Changed to</b> "Incubate overnight at 56°C."
27	27	<b>Section 3.1.3 (formerly 3.2.2)</b>	"Incubate for at least two hours in a 56°C oven."  <b>Changed to</b> "Incubate for at least two hours at 56°C."
28	28	<b>Section 3.1.3 (formerly 3.2.2)</b>	"Incubate overnight in a 56°C oven."  <b>Changed to</b> "Incubate overnight at 56°C."
55	55	<b>Section 9.1</b>	"Temperatures for refrigerators/freezer are recorded by the Forensic Biology Technician weekly, typically at the end of the week. Another individual may be assigned in the technician's absence."  <b>Changed to</b> "Temperatures for refrigerators/freezer are monitored electronically as a component of the laboratory security system."
55	55	<b>Section 9.1</b>	<b>Removed</b> "The acceptable temperature ranges for each unit is indicated at the top of the temperature log sheet."  and

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			"The temperature log templates are located at the end of this document."
-	63	9.7	<b>REMOVED</b> Maintenance information for Qiagen BioRobot M48 and renumbered the subsequent sections
74-75	--	<b>Section 9.9.2.1 And Section 9.10.2.1</b>	<p><b>ADDED</b> "The above test checks the resolution of the system. The allelic ladders will be run to perform the TH01 locus 9.3 and 10 alleles single band resolution check. The peaks will be deemed clearly defined if (i) both these peaks are above the analytical (reporting) threshold of 100RFU and (ii) both these allelic peaks are correctly called and labeled.</p> <ul style="list-style-type: none"> <li>• Injection "positive" control: Inspection of all the loci and all the allelic peaks at all the loci in the allelic ladder will be conducted to ensure that all standard peaks (Promega PowerPlex16 manual) are above the analytical threshold and correctly called and labeled.</li> <li>• Injection "negative" control: A mixture consisting of only Formamide and PowerPlex16 ILS in appropriate proportions (listed earlier) will be injected to ensure that (i) the ILS peaks are properly defined and labeled and are above the analytical/reporting threshold and (ii) no artifacts or spurious "allelic" peaks are observed above</li> </ul>

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			<p>the analytical/reporting threshold. (Alternatively or in addition to (C), a negative amplification control may also be run to perform the above test – but the first option is preferable since it checks the performance of the genetic analyzer components without introducing artifacts that may be PCR amplification related and not necessarily reflect a problem with the CE process).</p> <p>Failure to achieve the above expected results will require a repeat run and/or performance check and/or additional tests until the system demonstrates expected results.</p>
-	77	DNA Critical Reagent Verification Form	Removed M48 Extraction Kit and components
85- various	85-	Refrigerator/Freezer Temperature Logs	Removed all except one refrigerator in post-PCR lab. Updated make/model information on the one remaining refrigerator log.
87- various	85-	DNA Incubator Temperature Logs	Removed all except two ((1) 37°C and (1) 56°C), modified to be generic and filled in by hand with model and serial number.
--	106-107	M48 Maintenance Log and Performance Check Record	Removed