APPENDIX A – CONVERSION FACTORS AND OTHER USEFUL INFORMATION

I. CONVERSION FACTORS

One microgram = 1 µg = 1 x 10^{-6} g = 1000 ng

One nanogram = 1 ng = 1 x 10^{-9} g = 1000 pg

One picogram = 1 pg = 1 x 10^{-12} g = 1 x 10^{-3} ng

One liter = 1 L = 1000 mL

One milliliter = 1 mL = 1 x 10^{-3} (0.001) L = 1000 µL

One microliter = 1 µL = 0.001 mL = 1 x 10^{-3} mL = 1 x 10^{-6} L

II. CONCENTRATIONS

A. Concentration can be expressed several ways:

1. Weight percent = (mass A/total mass of solution) x 100

or simplified: Wt $\%_A$ = (g_A/100 mL of solution) x 100

2. Volume percent = (volume A/total volume of solution) x 100

or simplified: Volume $\%_A$ = ($volume_A$/100 mL of solution) x 100

3. Molarity (*M*): *M* = no. moles solute A/no. liters solution

= molecular weight of solute A in 1000 mL solution

where 1 mole of A = 1 gram formula weight of A

4. Normality (*N*) = no. MW/no. liters solution

where in acid-base reactions:

MW acid = weight of acid which reacts with 1 mole of OH^-
MW base = weight of base which reacts with 1 mole of H^+

The normality of a given reagent depends on the reaction in which it participates. (Example: 1 L of 1*M* H_3PO_4 which can have *N* = 1, 2 or 3 depending upon the reaction in which it is involved.) Because of ambiguities, the concept of normality is to be used carefully.

B. Examples:

1. Prepare 50% solution of polyethylene glycol (PEG):

Place 50 g of PEG in a flask and bring to a volume of 100 mL with H_2O.

Wt % PEG = (50 g/100 mL) x 100 = 50%

2. Prepare 100 mL of 2*M* HCl from a stock solution of 12*M* HCl. (The question is how much stock solution of 12*M* HCl is needed?)

Conc of Stock Soln = C_s = 12*M* Conc of Final Soln = C_f = 2*M*

Volume of Stock Soln = V_s = ? Volume of Final Soln = V_f = 0.1 L

$C_s \bullet V_s = C_f \bullet V_f$

$V_s = (C_f \bullet V_f)/C_s = 2(0.1)/12 = 0.2/12 = 0.0167$ L

$V_s = 0.0167$ L = 16.7 mL

Answer: Take 16.7 mL of 12*M* HCl and dilute to final volume of 100 mL to give 2*M* HCl.

APPENDIX B - REAGENTS

1. This appendix describes the preparation of reagents necessary for the DNA analysis. For each reagent listed, the company and catalog number is included. As a reagent is prepared, it will be labeled to include the following information:

   Identity
   Concentration
   Lot number
   Date of preparation
   Initials of preparer
   Date of expiration

   and if appropriate:

   Date of autoclaving
   Storage requirements

2. All reagents will be prepared with Type I water, unless otherwise stated.

3. All chemicals and reagents will be stored according to the manufacturers' specifications. All chemicals containing biologicals will be disposed of in biohazard bags. Unless otherwise stated, all other reagents used in Data Bank operations can be disposed of in the laboratory sink.

4. Any changes in chemical supply companies will be carefully checked by the Section Supervisor to ensure the chemical being provided meets the specifications necessary for the reagent. Any changes in chemical supply companies will be brought to the Forensic Biology Program Manager's attention so the list can be updated as necessary.

5. Concentrations preceding reagent components reflect the final concentrations of that specific component in the resulting mixture.

6. When a reagent is diluted and used by a number of analysts and retained, a lot number must be created and the reagent must be traceable to the reagent log. The container with the diluted reagent must contain the reagent stock lot number, the date the dilution was prepared, and the initials of the individual preparing the dilution. If a stock solution is diluted and used once and discarded or the dilution is used only by the analyst preparing the dilution, a new lot number does not need to be assigned or recorded in the reagent log. The original reagent stock lot number will be used.

ANODE BUFFER CONTAINER
Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Foster City, CA. PN 4393925

CATHODE BUFFER CONTAINER
Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Foster City, CA. PN 4408256

CONDITIONING REAGENT
Expiration date: Manufacturer's expiration date
Purchased from Applied Biosystems, Foster City, CA. PN 4409543

ETHANOL, 95%
Expiration date: Manufacturer's expiration date if listed, otherwise 3 years from date of receipt

HI-DI™ FORMAMIDE

Expiration date: Manufacturer's expiration date

Purchased from Applied Biosystems, Inc., Foster City, CA, PN 4311230, 25 mL each. Aliquot into 500 µL aliquots and store at -20ºC.

PERFORMANCE OPTIMIZED POLYMER – 4 (POP-4)

Expiration date: Manufacturer's expiration date

Purchased from Applied Biosystems, Foster City, CA. PN 4393715 (384 samples) OR PN 4393710 (960 samples)

PHOSPHATE-BUFFERED SALINE (PBS), 1X, pH 7.2

Expiration date: Manufacturer's expiration date if listed, otherwise 3 years from date of receipt

POWERPLEX® FUSION SYSTEM KIT

Expiration date: Manufacturer's expiration date

Purchased from Promega, Madison, WI, Catalog Number DC2402 for 200 reaction kit or DC2408 for 800 reaction kit.

Kit components included (expiration date same as kit unless specified):

- POWERPLEX® FUSION 5X MASTER MIX
- POWERPLEX® FUSION 5X PRIMER PAIR MIX
- WATER, AMPLIFICATION GRADE
- 2800M CONTROL DNA, 10 ng/µl
- POWERPLEX® FUSION ALLELIC LADDER MIX
- CC5 OR WEN INTERNAL LANE STANDARD 500

POWERPLEX® 5-DYE MATRIX STANDARDS, 3100/3130

Expiration date: Manufacturer's expiration date

Purchased from Promega, Madison, WI. Catalog Number DG4700.

POWERPLEX® 5C MATRIX STANDARD (contains WEN dye)

Expiration date: Manufacturer's expiration date

Purchased from Promega, Madison, WI. Catalog Number DG4850.

PUNCHSOLUTION™ KIT

Expiration date: Manufacturer's expiration date

Purchased from Promega, Madison, WI. Catalog Number DC9271.

QIAamp® DNA BLOOD MINI KIT

Expiration date: Refer to individual kit component expiration dates.

Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 51104 for the 50 sample kit OR 51106 for 250 sample kit.

Kit components include:

- QIAGEN® AL LYSIS BUFFER - Expiration date: Twelve months from date of receipt
- QIAGEN® AW1 WASH BUFFER (concentrate) - Expiration date: Twelve months from date of receipt. Dilute according to manufacturer's instructions before initial use.
- QIAGEN® AW2 WASH BUFFER (concentrate) - Expiration date: Twelve months from date of receipt. Dilute according to manufacturer's instructions before initial use.
- QIAGEN® AE ELUTION BUFFER – contained in the kit, but not used in Data Bank protocols
- QIAGEN® PROTEASE - Expiration date: Twelve months from date of receipt. Lyophilized protease may be stored at room temperature. Reconstitute with protease solvent before first use. After reconstitution with protease solvent, move bottle to 4 °C. Avoid prolonged periods at room temperature after reconstitution. If the QIAGEN® Protease is purchased separately from the kit, then it is to be reconstituted with 7 mL of Type I water for each 125 mg bottle.
- QIAGEN® PROTEASE SOLVENT - Expiration date: Twelve months from date of receipt

APPENDIX C - MAINTENANCE OF THE ABI 3500XL GENETIC ANALYZER

1 The weekly and monthly maintenance requirements for the 3500xl are specified in Table 1.

Task	Frequency
Restart the computer and instrument	At each polymer change or as needed
Use a lab wipe to clean the anode buffer container valve pin assembly on the polymer delivery pump	Monthly or as needed
Run the Wash Pump and Channels Wizard (conditioning reagent)	Monthly or as needed
Flush the pump trap	Monthly or as needed
Empty the water trap waste container. The waste container is to the right of the pump block	Monthly or as needed
Replace cathode buffer septa	Monthly or as needed
Clean the autosampler and drip tray	Monthly or as needed
Remove dried polymer from the capillary tips with a lint-free wipe moistened with deionized water	Monthly or as needed
Defragment the hard drive	Monthly or before fragmentation reaches 10%

Table 1. Maintenance Tasks

1.1 Daily or pre-run tasks are specified in Section 3.6.8 of this manual

2 Wizards - Wizards are automated instrument processes which allow the user to perform a variety of tasks. Each wizard has been designed with specific instructions to achieve the purpose of the wizard. The wizards are accessed by selecting the desired wizard from the "Maintenance Wizards" menu.

The wizards include:

2.1 Install a capillary array

Select Install Capillary Array wizard. Follow directions given in the wizard to change or install a capillary array. This wizard will take 15 to 45 minutes to complete.

2.2 Remove bubbles from the polymer pump

To remove bubbles from the polymer pump fluid path that travel from the polymer pouch through the pump, array port, and the anode buffer container, select Remove Bubbles wizard. Follow the directions given in the wizard. This wizard will take 5 to 15 minutes to complete.

2.3 Wash the pump chamber and channels

2.3.1 Conditioning reagent is used for priming the polymer pump, washing the polymer pump between polymer type changes, and during instrument shut down. Each pouch has adequate volume for a one-time use.

2.3.2 Select Wash pump chamber and channels wizard. Follow directions given in the wizard to wash the pump chamber and channels. The procedure takes about 25 minutes to complete.

2.3.3 Record the date of the conditioning reagent run, lot number and expiration of the reagent pouch in the appropriate QC log.

2.4 Fill the array with polymer

To fill the capillary array with the same type of polymer, in the Data Collection Software, select Maintenance Wizards>Fill the Array with fresh Polymer. Follow the directions given in the wizard.

2.5 Replenish the polymer installed on the instrument

2.5.1 Select Replenish Polymer Wizard. Follow the directions given in the wizard to load fresh polymer on the instrument. This wizard takes 10 to 20 minutes to complete.

2.5.2 Allow the new polymer pouch to equilibrate to room temperature prior to installing on the instrument. Verify that the polymer has not expired as the instrument WILL allow you to install polymer that is past the manufacturer's expiration.

2.5.3 Click "Refresh" on the dashboard to update the screen.

2.5.4 Record the date, lot number, and expiration date on the 3500*xl* Reagent Log.

2.6 Change the type of polymer installed on the instrument

All DFS DNA Data Bank validated procedures use POP4. This wizard will not be used.

2.7 Shutdown the instrument

For short-term and long-term shutdown, select Shutdown the instrument wizard. Follow the directions given in the wizard. This wizard takes 60 minutes to complete.

2.8 Reactivate the instrument

For use after a period of inactivation - this wizard assumes that the Instrument Shutdown wizard was used previously. The pump chamber and channels are filled with conditioning reagent, and no array is installed.

3 Restarting the computer

The computer and instrument will both be shut down and restarted each time the polymer is changed (Replenish polymer wizard). This action will be recorded on the 3500xl Reagent Log.

4 Defragment the hard drive

Go to Start>Programs>Accessories>System Tools>Disk Defragmenter and follow the prompts.

4.1 You can click "Analyze" to see if you should defragment or not.

5 Changing the Anode Buffer Container (ABC)

5.1 The lot number of the ABC is recorded in the raw data of each electropherogram. Verify that the buffer level is at or above the fill line and check that the seal is intact.

5.2 Invert the ABC, and then tilt it slightly to make sure most of the buffer is in the larger side of the container. There should be less than 1 ml of the buffer remaining in the smaller side of the container.

5.3 Peel off the seal at the top of the ABC and place the ABC into the Anode end of the instrument.

5.4 Click "Refresh" on the dashboard to update the screen.

6 Changing the Cathode Buffer Container (CBC)

6.1 The lot number of the CBC is recorded in the raw data of each electropherogram. Press the Tray button on the instrument to bring the old CBC forward. Remove the septas from the old container prior to discarding.

6.2 Verify that the buffer level is at or above the fill line and check that the seal is intact.

6.3 Tilt the CBC back and forth gently to ensure that the buffer is evenly distributed across the top of the baffles.

6.4 Peel off the seal at the top of the CBC. Wipe off any buffer to make sure it is dry.

6.5 Align and place the appropriate septa both sides. Push the septa lightly into the holes to start and then push firmly to seat the septa. Install the CBC on the autosampler.

6.6 Click "Refresh" on the dashboard to update the screen.

7 Flush the water trap (pump trap)

7.1 The water trap must be flushed once per month to prolong the life of the pump and to clean any diluted polymer.

7.2 Fill a 20mL, Luer lock syringe with distilled or deionized water. Expel bubbles from the syringe. Do not use a syringe smaller than 20mL as the pressure generated will be too great.

7.3 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 with the other hand.

7.4 Open the Luer fitting by grasping the body of the fitting and turning it to loosen. Turn counterclockwise approximately one-half turn.

7.5 DO NOT USE EXCESSIVE FORCE when you push the syringe plunger as this may damage the trap seals. Take approximately 30 seconds to flush 5 mL of water through the trap.

7.6 Remove the syringe from the Luer fitting. Hold the fitting with one hand while turning the syringe counterclockwise with the other hand.

7.7 Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.

8 Performing a spatial

8.1 A spatial calibration is used by the instrument to correlate the signal from each capillary with the signal detected by the CCD camera. A spatial must be performed after any of the following events:

- Replaced the capillary array
- Opened the detector door or moved the detection cell
- Moved the instrument
- Service engineer performed an optical service procedure, such as realigned or replaced the laser or CCD camera or mirrors on the instrument.

8.2 To access the Spatial Calibration screen, select Maintenance>Spatial Calibration in the navigation pane.

8.3 Select "No Fill" or "Fill" to fill the array with polymer before starting the calibration.

8.4 Check "Perform QC checks". The software will check each capillary against the specified range for spacing and intensity.

8.5 Click Start Calibration. The display updates as the run progresses.

8.6 A Spatial QC Check error message is displayed if:

- The average peak height or individual peak height is below the threshold
- Uniformity or capillary spacing exceeds the threshold

8.7 When the run is complete, evaluate the calibration profile to ensure that you see:

- One sharp peak for each capillary. Small shoulders are acceptable
- One marker (+) at the apex of every peak. No off-apex markers.
- An even peak profiles (all peaks about the same height)

8.8 If the results meet the above criteria, click Accept Results. An example of a successful spatial profile is seen in Figure 2.

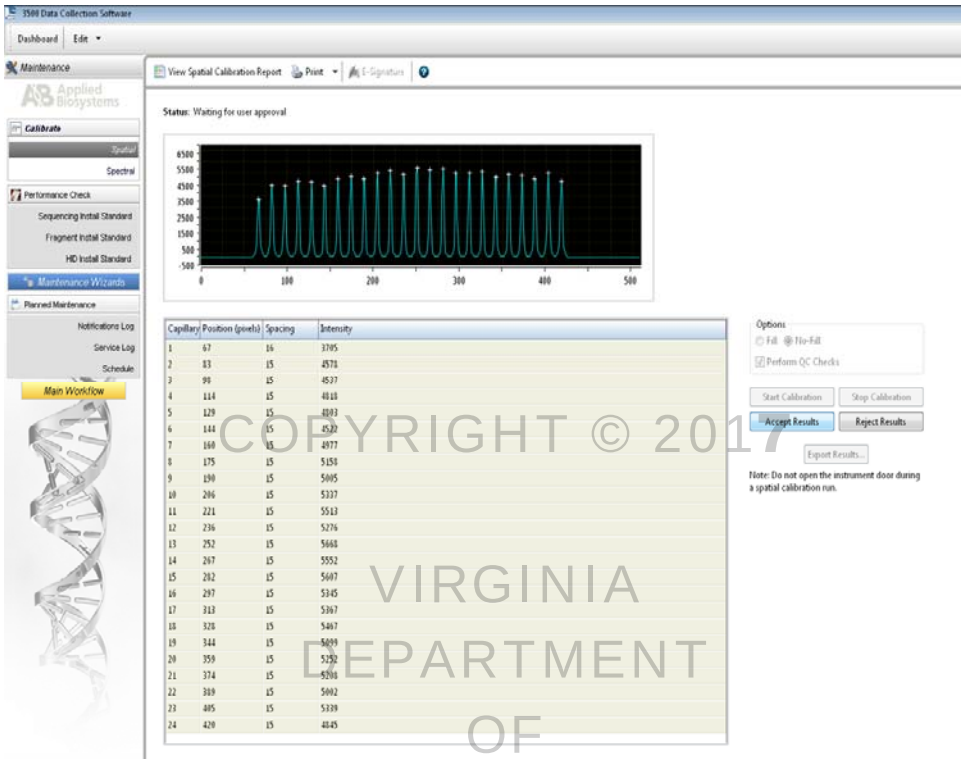

Figure 2. An example of an acceptable spatial calibration profile.

8.9 If the results do not meet the above criteria, click Reject Results, then go to "Spatial Calibration Troubleshooting" in the Applied Biosystems ®3500xl Genetic Analyzer user guide.

8.10 Only the most recent spatial calibration is maintained in the software. The software does not save historical calibration results.

9 Performing a spectral

9.1 The spectral calibration should be performed on dye set G5. Once generated, this file is applied during sample detection to calculate the spectral overlap between the five different dyes and separate the raw fluorescent signals into individual dye signals.

9.2 Perform a spectral for each dye set/polymer type combination you will use. A spectral must also be performed after any of the following events:

- Replaced the capillary array
- If a decrease is seen in spectral separation (pull-up in peaks) in the raw or analyzed data
- Service engineer performed an optical service procedure, such as realigned or replaced the laser or CCD camera or mirrors on the instrument

9.3 Refer to the appropriate Promega technical protocol for specific spectral instructions.

10 Long periods of inactivity

If the ABI 3500*xl* Genetic Analyzer remains unused for an extended period of time (approximately 2 weeks or longer), it is recommended that either a blank run (consisting of only formamide and size standard) be completed *or* the capillary array removed and the capillary ends stored in Type I H_2O until further use.

APPENDIX D – STORAGE OF ELECTRONIC DATA

Saving Sample files and Project files

1 Sample files and project files generated by the data bank using the Applied Biosystems, Inc. 3500*xl* Genetic Analyzer and analyzed using the GeneMapper® *ID-X* software will be saved onto a storage medium and stored according to the following procedures.

2 After the data has been technically reviewed and found to be acceptable, the electronic sample files and project files will be transferred onto a medium for permanent storage. The storage medium will be labeled appropriately to indicate the data files are PowerPlex® Fusion samples, the appropriate plate number, date and analyst's initials. The folders may be deleted from the computer after the data has been stored and a backup copy has been made.

3 The storage medium will then be stored securely with the data bank sample plate documentation in a specified location within the laboratory for easy retrieval.

4 If a file(s) needs to be subsequently added to the storage medium for a given plate of data bank samples, the analyst will make arrangements with his/her supervisor to have the storage medium retrieved from storage and have the file(s) added to the appropriate storage medium.