Procedure for DNA Reagent Preparation and Quality Control

1.0 Purpose - This procedure specifies the required elements for the preparation of, and quality control procedures for, reagents used in the Forensic Biology Section.

2.0 Scope – This procedure applies to all Forensic Scientists in the Forensic Biology Section.

3.0 Definitions

- **Commercial Reagent**: A commercially produced laboratory reagent designed to conduct a specific forensic test. All commercial reagents shall have an expiration date either established by the manufacturer or, if none is provided, the Forensic Biology Section shall establish the expiration date.
  Commercial reagents in the Forensic Biology Section: ProK (both stock supply and aliquots), ProK from DNA Investigator Kit, DTT (both stock supply and aliquots), Hi-Di formamide (both stock supply and aliquots), 20 % SDS, 10x buffer, 1x buffer, nuclease-free dH_2O, LIZ sizing standard, spectral/matrix kits for 3130XL (or equivalent) and 7500 (or equivalent), Carrier RNA (cRNA) from the DNA Investigator Kit.
  See **5.4** for specific expiration dates. Expiration dates shall be written on bottles/containers by the Section employee who receives the commercial reagent. If multiple reagents are received, the expiration date may be written on a parent container (e.g., formamide, LIZ, nuclease-free dH_2O).
- **Critical Reagent:** Determined by empirical studies or routine practice to require reliability testing on established samples before use on evidentiary samples. All critical reagents shall have an expiration date as established by the manufacturer or the Forensic Biology Section.
  Critical reagents in the Forensic Biology Section: STR-Tris-EDTA (STR-TE), STR-Stain Extraction Buffer (STR-SEB), commercially supplied kits and their components (AmpF*l*STR® Identifiler Plus®, Quantifiler® Duo, DNA Investigator).
- **QCO**: Refers to the DNA Quality Control Officer and/or his/her designee.
- **Limited Access:** A location to which not all Forensic Scientists, Supervisors, or Managers have access.

4.0 Equipment, Materials and Reagents

- pH test strips (see Forensic Biology Section Procedure for Body Fluid Unit Quality Control)
- Chemicals: concentrated hydrochloric acid (HCl), sodium hydroxide pellets (NaOH), ethylenediaminetetraacetic acid, granular (EDTA), sodium chloride, granular (NaCl), sodium dodecyl sulfide (SDS), Trizma base (Tris), glycogen, glacial acetic acid, sodium acetate anhydrous
- Nuclease-free distilled water (nuclease-free dH_2O)
- Distilled water (dH_2O) from in-house filtered water supply system
- Certified Biosafety Cabinet and/or certified chemical fume hood
- Various lab equipment (lab tape, autoclave tape, Alconox (or equivalent), Kimwipes, pipettes and associated tips, cleaned and sterilized glassware, heat/stir plate, vacuum pump, magnetic stir bars, 96-well trays and septa, amplification trays, pH buffers)

5.0 Procedure

5.1 NIST SRM/ Standard Traceable to NIST

5.1.1 **Purpose and Use:** The QCO shall test the analytical procedures used against the appropriate National Institute of Standards and Technology (NIST) Standard Reference Material (SRM), or Standard Traceable to NIST (NIST-TS), on an annual basis. The NIST SRM or NIST-TS shall also be tested when substantial changes, new procedures, or new platforms are validated in these units, as well as against commercially produced kits.

5.1.2 **Creating a Standard Traceable to NIST:** The QCO shall create a batch of known human bloodstains from a male individual whose DNA profile has been previously established as follows:

5.1.2.1 Dispense liquid blood from donor onto several sheets of FP705 paper (or equivalent) until all collected liquid blood is deposited and allowed to dry completely.

5.1.2.2 A sample from this batch of bloodstains shall then be extracted (using current Forensic Biology Section extraction procedure(s)) along with an associated negative extraction control (Neg K), as well as any part of the NIST SRM that requires extraction (i.e., SRM's E and F in NIST SRM 2391c).

5.1.2.3 This extracted bloodstain and Neg K, as well as any extracted NIST SRMs shall then be quantitated, amplified, electrophoresed and analyzed simultaneously, along with any NIST SRMs which may have already been supplied in liquid form (i.e., NIST SRMs A-D in NIST SRM 2391c) according to applicable Forensic Biology Section DNA procedures.

5.1.2.4 The bloodstain and all NIST SRMs shall provide the expected allele calls, and all testing negatives (including Neg K) shall be free of any alleles. If either condition is not met (for reasons other than instrument failure or known artifacts), then the QCO may retest the bloodstain and/or NIST SRMs once. If both conditions are not met this second time, a new lot of bloodstains and/or NIST SRM shall be tested.

5.1.2.5 Once the conditions in **5.1.2.4** are met (i.e., the expected allele calls are obtained and the testing negatives are free of any alleles), this batch of bloodstains shall be accepted as a suitable NIST-TS and the entire lot of bloodstains shall be named/referred to by the initials of the blood donor, followed by the date on which the bloodstains were prepared (e.g., XXX_12012010). The QCO shall document the testing performed and retain such documentation in the Section, along with the NIST SRM documentation provided by the manufacturer.

5.1.2.6 If other testing kits become available for use in the Forensic Biology Section, the appropriate NIST SRM for that kit shall be tested against a batch of known human bloodstains from a male individual. This batch may be the same NIST-TS currently in use if enough of that batch remains available for testing.

5.1.3 **Storage:** The NIST SRM shall be stored long-term at -20 °C with limited access by the QCO; the NIST-TS (bloodstains) shall be stored with limited access by the QCO at room

temperature; extracted NIST-TS (liquid form) and associated Neg K shall be stored at 4 °C for up to 1 year after date of approval for use by the DNA Technical Leader with limited access by the QCO for use in QC testing. After 1 year, these extracts shall be discarded by the QCO.

5.2 Preparation and QC of Reagents/Solutions/Standards

5.2.1 Naming/Recording of Reagents/Solutions/Standards:

5.2.1.1 The following items shall be recorded in Forensic Advantage (FA) under the Resource Manager by the QCO as follows: Item description expiration date (e.g., 0.5M EDTA_06082011):

- 0.5M EDTA
- 1M Tris-HCl
- 20 % SDS
- STR-SEB
- STR-TE
- STR-Tris-EDTA-NaCl (STR-TEN)
- 3M NaOAc pH 5.0

5.2.1.2 The following items shall be recorded in FA under the Resource Manager by the QCO as follows: Item lot number expiration date (e.g., A9815D0209_03152011):

- ProK (aliquots)
- DTT (aliquots)
- Formamide (aliquots)
- Carrier RNA (aliquots)
- Any item listed in **5.2.1.1** or **5.2.1.2** if purchased directly from manufacturer

5.2.1.3 The following items shall be recorded in FA under the Resource Manager by the QCO based upon the lot numbers provided by the manufacturer. Any expiration dates (if applicable) shall be noted within the individual lot Resource Instance Details:

- Kits (Identifiler Plus®, Quantifiler® Duo, DNA Investigator)
- Kit components (e.g. reaction mix, primer, Taq, DNA standard, allelic ladder, positive and negative amplification controls, Carrier RNA, ProK)
- 3130XL POP-4, 10X buffer
- ProK, DTT, Hi-Di formamide, nuclease-free dH_2O (stock)
- SDS, HCl, EDTA, NaOH, NaCl, Tris base

5.2.2 For all items which require testing for reliability (QC check), the date on which the item passes Quality Control (QC) shall be entered into FA under the "date verified" line by the QCO performing the QC check.

5.2.3 Documentation: Any documentation generated from the preparation or QC check of any reagents, kits or standards shall be documented by the QCO in the QC files and thereafter maintained in the Section.

5.2.4 Solution/Reagent/Standards Preparation and QC (as noted):

Note: Glass bottles used in the preparation and storage of buffers and components shall be cleaned with Alconox (or equivalent), rinsed with dH_2O and autoclaved prior to use (see Forensic Biology Section Procedure for Aseptic Technique and Contamination Control).

5.2.4.1 0.5 M EDTA

Chemical/Reagent	Amount (for 500 mL)	Amount (for 1L)
EDTA	93.0 g	186.1 g
dH_2O	400 mL	800 mL
NaOH pellets	As needed	As needed

5.2.4.1.1 Verify pH test strips using known buffers.

5.2.4.1.2 Add EDTA to dH_2O.

5.2.4.1.3 Add NaOH pellets to get EDTA into solution (may take several pellets; add individually and wait several minutes to dissolve before testing pH and determining whether additional pellets are necessary). Use a magnetic stir bar on a stir/hot plate to mix EDTA. Heat may also be used to aid dissolution if kept on lowest setting.

5.2.4.1.4 Adjust to pH 8.0 (±0.3) with additional NaOH pellets (may require several pellets; add individually and wait several minutes to dissolve before testing pH) and evaluating for pH with test strips.

5.2.4.1.5 Adjust volume to 1 L (or 500 mL) once EDTA has gone into solution and pH 8.0 (±0.3) has been achieved.

5.2.4.1.6 Filter-sterilize with a 75 mm Nalgene filtration unit (or equivalent) using vacuum suction.

5.2.4.1.7 0.5 M EDTA shall be stored at 4 °C and discarded 6 months after date of preparation. Record preparation information in FA.

5.2.4.2 1 M Tris-HCl

Chemical/Reagent	Amount (500 mL)	Amount (1L)
Tris base	60.6 g	121.2 g
dH_2O	400 mL	800 mL
Concentrated HCl	~22.5 mL	~46 mL

5.2.4.2.1 Verify pH test strips using known buffers.

5.2.4.2.2 Add Tris base to dH_2O. Adjust pH to 8.0 (±0.3) by adding HCl, slowly and evaluating for pH with test strips. CAUTION: HCl is extremely corrosive. Use a magnetic stir bar on stir/hot plate to mix solution.

5.2.4.2.3 Bring to final volume with dH_2O.

5.2.4.2.4 Autoclave.

5.2.4.2.5 1 M Tris-HCl shall be stored at room temperature and discarded 6 months after date of preparation. Record preparation information in FA.

5.2.4.3 20 % SDS

Chemical/Reagent	Amount (500 mL)	Amount (1L)
Sodium dodecyl sulfate (SDS)	100 g	200 g
dH_2O	400 mL	800 mL

5.2.4.3.1 Dissolve SDS in dH_2O. To aid with dissolution, solution may be heated (lowest setting) and stirred using a magnetic stir bar on stir/hot plate.

5.2.4.3.2 Adjust to final volume with dH_2O.

5.2.4.3.3 Autoclave.

5.2.4.3.4 If the 20 % SDS falls out of solution (i.e., appears cloudy), that batch may be used if approved by the DNA Technical Leader, and shall be stored at 37 °C to keep the SDS in solution.

5.2.4.3.5 20 % SDS shall be stored at room temperature (unless **5.2.4.3.4** applies) and discarded 6 months after date of preparation. Record preparation information in FA.

5.2.4.4 STR-TE

Chemical/Reagent	Amount (for 1L)
1 M Tris-HCl	10 mL
0.5 M EDTA	200 µL
dH_2O	990 mL

5.2.4.4.1 Add EDTA to dH_2O.

5.2.4.4.2 Add Tris-HCl to dH_2O.

5.2.4.4.3 Using magnetic stir bar and stir/hot plate, mix together for 5 minutes.

5.2.4.4.4 Autoclave.

5.2.4.4.5 STR-TE shall be stored at room temperature and discarded on the date either the Tris-HCl or EDTA expires, whichever is earlier. Record preparation information in FA.

5.2.4.4.6 QC Testing:

5.2.4.4.6.1 A sample of the NIST-TS and Neg K shall be extracted, quantitated, amplified, electrophoresed, and analyzed according to applicable Section DNA Procedures.

5.2.4.4.6.2 The new lot of STR-TE shall be used at all steps which require the addition or use of STR-TE (after extraction).

5.2.4.4.6.3 The expected results for the NIST-TS shall be obtained for all loci and the alleles shall be balanced within and between loci and peak heights generally between 1000 and 6000 RFU's. The Neg K shall be free of any alleles. If either condition is not met (for reasons other than instrument failure or known artifacts), then the QCO may retest the new lot of STR-TE only once. If either condition is not met this second time, a new lot of STR-TE shall be prepared and tested.

5.2.4.5 STR-SEB

Chemical/Reagent	Amount (for 1 L)
NaCl	5.84 g
1 M Tris-HCl	10 mL
0.5 M EDTA	20 mL
dH_2O	~500 mL
20 % SDS	100 mL

5.2.4.5.1 Verify pH test strips using known buffers.

5.2.4.5.2 Add NaCl, EDTA, and Tris-HCl to ~500 mL of dH_2O until dissolved using a magnetic stir bar on the stir/hot plate. Slight heat (lowest setting) may be used to aid in dissolution.

5.2.4.5.3 Adjust to pH 8.0 (±0.3) with approximately 1 pellet of NaOH (if more are necessary, add only one at a time and allow it to dissolve completely before retesting the pH). Evaluate pH with test strips.

5.2.4.5.4 Add SDS.

5.2.4.5.5 Bring to final volume with dH_2O.

5.2.4.5.6 Autoclave.

5.2.4.5.7 STR-SEB shall be stored at room temperature and discarded on the date the Tris-HCl, EDTA, or SDS expires, whichever is earlier. If the 20 % SDS falls out of solution (see **5.2.4.3.5**), then the STR-SEB shall be kept at 37 °C, including any aliquots, in order to keep the SDS in solution. Record preparation information in FA.

5.2.4.5.8 QC Testing:

5.2.4.5.8.1 A sample of the NIST-TS and Neg K shall be extracted, quantitated, amplified, electrophoresed, and analyzed according to applicable Forensic Biology Section DNA Procedures.

5.2.4.5.8.2 The new lot of STR-SEB shall be used at the extraction step.

5.2.4.5.8.3 The expected results for the NIST-TS shall be obtained for all loci and the alleles shall be balanced within and between loci and peak heights generally between 1000 and 6000 RFU's. The Neg K shall be free of any alleles. If either condition is not met (for reasons other than instrument failure, known artifacts), then the QCO may retest the new lot of STR-SEB only once. If either condition is not met this second time, a new lot of STR-SEB shall be prepared and tested.

5.2.4.6 STR-TEN

Chemical/Reagent	Amount (for 1 L)
NaCl	5.84 g
1 M Tris-HCl	10 mL
0.5 M EDTA	2 mL
dH_2O	~ 700 mL

5.2.4.6.1 Verify pH test strips using known buffers.

5.2.4.6.2 Add NaCl, EDTA, and Tris-HCl to the dH_2O and stir until dissolved.

5.2.4.6.3 Adjust to pH 8.0 (±0.3) with approximately 1 pellet of NaOH. Evaluate pH with test strips.

5.2.4.6.4 Bring to final volume with dH_2O.

5.2.4.6.5 Autoclave.

5.2.4.6.6 STR-TEN shall be stored at room temperature and discarded on the date either the Tris-HCl or EDTA expires, whichever is earlier. Record preparation information in FA.

5.2.4.6.7 QC Testing:

5.2.4.6.7.1 A known semen (containing sperm) stain shall be extracted via differential extraction; only the sperm fraction and associated control is required to be carried through QC testing (e.g., quantitated, amplified, electrophoresed and analyzed) according to applicable Forensic Biology Section DNA Procedures.

5.2.4.6.7.2 The expected results for the known male contributor shall be obtained for all loci and the alleles shall be balanced within and between loci and peak heights generally between 1000 and 6000 RFU's. The negative extraction control shall be free of any alleles. If either condition is not met (for reasons other than instrument failure, known artifacts), then the QCO may retest the new lot of STR-TEN only once. If either condition is not met this second time, a new lot of STR-TEN shall be prepared and tested.

5.2.4.7 0.39M STR-Dithiothreitol (DTT)

Chemical/Reagent	Amount (for 10 mL)	Amount (for 25 mL)
Dithiothreitol (McCleland's reagent, stock)	601 mg	1.5 g
Sterile nuclease-free dH_2O	10 mL	25 mL

5.2.4.7.1 Add water to DTT to reconstitute and mix well.

5.2.4.7.2 Aliquot 200 µL into sterile yellow-colored 0.5 mL tubes while under a Biological Safety Cabinet (or equivalent).

5.2.4.7.3 Freeze aliquots immediately at -10 °C. Once aliquot is thawed it shall not be refrozen and after use, the remainder of the aliquot shall be discarded. The master supply of aliquots shall be stored at -20 °C; working stock supplies of DTT shall be kept at -10 °C.

5.2.4.7.4 See **5.2.1.2** for naming convention and FA entry.

5.2.4.7.5 Aliquots expire 1 year after date of reconstitution, or when stock supply expires, whichever occurs first.

5.2.4.8 Hi-Di Formamide

5.2.4.8.1 The QCO shall thaw formamide to 4 °C and aliquot 180 µL (or multiples of 180 µl) into autoclaved clear 1.5 mL sterile tubes for casework.

5.2.4.8.2 The aliquots shall be frozen immediately at -10 °C. Once aliquot is thawed it shall not be refrozen, and after use the remainder of the aliquot shall be discarded by the Forensic Scientist. Aliquots expire 1 year after date of preparation, or when stock supply expires, whichever occurs first.

5.2.4.8.3 See **5.2.1.2** for naming convention and FA entry.

5.2.4.9 3M Sodium Acetate pH 5.0 (3M NaOAc)

Chemical/Reagent	Amount (for 100 mL)
Sodium acetate anhydrous	**24.6 g**
dH2O	**80 mL**
Glacial acetic acid	**As needed**

5.2.4.9.1 Dissolve the sodium acetate in dH2O. To aid with dissolution, solution may be heated (lowest setting) and stirred using a magnetic stir bar on stir/hot plate.

5.2.4.9.2 Once all the salts have dissolved, adjust the pH to 5.0 with glacial acetic acid. Evaluate the pH level with test strips.

5.2.4.9.3 Bring to final volume with dH2O.

5.2.4.9.4 Autoclave.

5.2.4.9.5 3M NaOAc shall be stored at room temperature and discarded 6 months after date of preparation. Record preparation information in FA.

5.2.4.10 Carrier RNA

Chemical/Reagent	Amount
Carrier RNA	**310 µg (one tube)**
Sterile nuclease free dH2O	**310 µL**

5.2.4.10.1 Add water to RNA to reconstitute and mix well.

5.2.4.10.2 Aliquot 50 µL into sterile purple colored 0.5 mL tubes while under a Biological Safety Cabinet (or equivalent).

5.2.4.10.3 Freeze aliquots immediately at -10 °C. Once aliquot is thawed it shall not be refrozen and after use. The remainder of the aliquot shall be discarded. The master supply of aliquots shall be stored at -20 °C; working stock supplies of RNA shall be kept at -10 °C. Aliquots expire 1 year after date of preparation, or when stock supply expires, whichever occurs first.

5.2.4.10.4 See **5.2.1.2** for naming convention and FA entry.

5.2.4.11 DNA Quantitation Standards

5.2.4.11.1 The QCO shall prepare Standard "A" as described below. The Forensic Scientist shall prepare the remaining standards as needed (serial dilution) using only the Quantifiler® Duo DNA Dilution Buffer and the DNA standards provided in the Quantifiler® Duo Kits. Volumes may be adjusted by the Forensic Scientist as long as the dilution factor remains constant.

5.2.4.11.2 Each standard shall be mixed thoroughly and centrifuged before proceeding to the next standard.

5.2.4.11.3 The standards shall be prepared every 60 days, or as needed until their expiration date. The standards shall be prepared in sterile 1.5 mL clear plastic tubes.

5.2.4.11.4 Standards "A" and "H" have higher volumes when preparation is complete.

5.2.4.11.5 Quantitation standards shall be stored at 4 °C.

Standard	**Amount of Quantifiler Duo DNA Dilution Buffer (in µL)**	**Amount of Standard (in µL)**
1 ("A")	600	200 (stock)
2 ("B")	400	200 (Standard 1)
3 ("C")	400	200 (Standard 2)
4 ("D")	400	200 (Standard 3)
5 ("E")	400	200 (Standard 4)
6 ("F")	400	200 (Standard 5)
7 ("G")	400	200 (Standard 6)
8 ("H")	400	200 (Standard 7)

5.3 QC of Commercial Kits

5.3.1 **AmpF*l*STR® Identifiler Plus:** the performance of each lot of Identifiler Plus shall be checked by the QCO against the NIST-TS as described below prior to use in the Forensic Biology Section.

5.3.1.1 The following items shall be quantitated (only required for 9947A), amplified, electrophoresed and analyzed according to applicable Forensic Biology Section DNA Procedures:

5.3.1.1.1 Standard Traceable to NIST and associated Neg K (previously extracted, if available).

5.3.1.1.2 9947A (positive amplification control).

5.3.1.1.3 Negative Amplification Control.

5.3.1.2 Both the Standard Traceable to NIST and 9947A shall produce the expected results at all loci tested. Alleles shall be balanced within and between loci and peak heights generally between 1000 and 6000 RFUs.

5.3.1.3 The Neg K and negative amplification control shall not exhibit any alleles.

5.3.1.4 The allelic ladder associated with each new lot of Identifiler Plus shall produce the correct expected alleles.

5.3.1.5 If the kit fails to meet either **5.3.1.2**, **5.3.1.3**, or **5.3.1.4** (for reasons other than instrument failure, known artifacts), it may be retested once. If the kit fails this second re-test, it shall not be accepted for any use in the Section and the DNA Technical Leader and kit manufacturer shall be notified immediately by the QCO.

5.3.1.6 The kit information (lot numbers, date verified, and expiration date) and 9947A concentration shall be entered into the FA system per **5.2.1.3** by the QCO.

5.3.1.7 The general supply of Identifiler Plus kits shall be stored at -20 °C by the QCO; active working stock shall be kept at 4 °C.

5.3.2 **Quantifiler® Duo:** the performance of each lot of Quantifiler® Duo shall be evaluated by the QCO as described below. The same ABI 7500 shall be used throughout the evaluation. The QCO shall use the pipettes designated for QC purposes only. Only the human portion of the resulting quantitation data shall be used for assessing data quality throughout the process. Variation should be of no more than 2 C_t of the IPC values between the results obtained in validation (for equivalent standards/samples) at all stages of QC testing. Each new lot of Quantifiler® Duo DNA standard shall be calibrated against NIST SRM 2372.

5.3.2.1 **Kit Component QC Testing:** Prior to calibration of the new DNA Standard, the components of the new lot of Quantifiler® Duo (PCR reaction mix and primer) shall be tested against the current lot of Quantifiler® Duo DNA Standard.

5.3.2.1.1 Master mix using the new kit reagents (reaction mix and primer) shall be prepared and used for a plate containing the following: a standard curve (in duplicate), at least one NTC, one Standard Traceable to NIST and the associated negative extraction control(s) for the Standard Traceable to NIST (if applicable).

5.3.2.1.2 All negative control(s) shall be free of DNA or have an IPC of ≥ 36. All Standards Traceable to NIST shall indicate the presence of DNA.

5.3.2.1.3 The standard curve shall have acceptable quality metrics as follows: slope (-3.00 to -3.60), R^2 (≥ 0.98). The y-intercept shall be +/- 0.25 of that obtained in the original validation.

5.3.2.1.4 If these criteria are not met, the test may be repeated upon the authority and direction of the DNA Technical Leader. If the criteria are successfully met, the new lot of PCR reaction mix and primer shall be used for the remaining portions of the quality control check of the new Quantifiler® Duo kit.

5.3.2.2 **DNA Standard Calibration:** New lots of Quantifiler® Duo DNA standard shall be calibrated against NIST SRM 2372 as follows:

5.3.2.2.1 Using components A, B, and C from the SRM, prepare a standard curve for each with a starting concentration of 50 ng/µl and serially dilute as described in 5.2.4.11 for a total of eight quantities per component. Use the Dilution Buffer from the currently acceptable Quantifiler Duo kit to prepare the standard curved. Note: volumes may be reduced in scale in order to prepare the standard curves.

5.3.2.2.2 Prepare two independent serial dilution sets (A and B) according to the table below with the new lot of Quantifiler® Duo DNA standard:

	Volume	Quant Duo Dilution Buffer	Dilution Factor
1:20	9 µl of stock	171 µl	1:20
1:60	30 µl of 1:20	60 µl	1:3
1:180	30 µl of 1:60	60 µl	1:3
1:540	30 µl of 1:180	60 µl	1:3

5.3.2.2.3 Using the PCR reaction mix and primer from the new Quantifiler® Duo kit, prepare a plate as follows: run the standard curve for each NIST SRM component as described in **5.3.2.2.1**, for a total of 3 curves, and both serial dilutions sets (A and B). Each serial dilution from both sets shall be run in triplicate, at a minimum. An NTC shall also be included on the plate, at a minimum. Within the HID software on the 7500 instrument, designate the NIST SRM curves as DNA standards and label each with the appropriate expected quantities. The dilution set samples shall be left as unknowns.

5.3.2.2.4 Verify that the NIST SRM standard curves both independently, and then combined, result in slopes and R^2 values within acceptable ranges. The y-intercepts between each of the three curves must be +/- 0.25 of each other in order for the curves to be combined. If one of the three SRM curves is out of specification, the QCO may prepare that particular curve a second time or omit it from further analysis with the written approval of the DNA TL.

5.3.2.2.5 Using the combined NIST SRM standard curves, analyze the serially diluted samples. Determine the approximate DNA concentration of the new lot of Quantifiler® Duo DNA standard by multiplying the quant value obtained for each dilution by its dilution factor (e.g., if the quantity obtained is 1.5 ng/µl and the dilution factor is 20, then the total ng quantified is 30 ng/µl).

5.3.2.2.6 Average all derived concentrations from the previous step to determine the approximate quant value of the new lot of Quantifiler® Duo DNA standard.

5.3.2.3 Standard Curve QC Testing: New lots of calibrated Quantifiler® Duo DNA standards shall be evaluated for satisfactory quality metrics prior to passing QC.

5.3.2.3.1 Using the previously determined approximate concentration of the new lot of Quantifiler® Duo DNA standard (see 5.3.2.2), prepare a

standard curve dilution series (Standards A through H, 50 ng/µl to 0.023 ng/µl). Refer to 5.2.4.11 for preparation of the standard curve.

5.3.2.3.2 Run the standard curve (in duplicate) on the same plate with the NIST SRM prepared standard curves (each component singly). Use the PCR reaction mix and primer from the new kit.

5.3.2.3.3 On the same plate, include at least one negative template control (NTC) and at least one Standard Traceable to NIST (run in triplicate).

5.3.2.3.4 Assess the quality metrics of the standard curves (combine the NIST SRM standard curves); the slope and R^2 values for each must meet previously specified criteria. Compare the y-intercepts of the NIST SRM standard curve to the new standard curve; the y-intercepts shall be within +/- 0.25 of each other in order for this portion of the QC testing to pass. Also, average the IPC Ct values for each standard curve and compare the average between the new DNA standard and that of the NIST SRM standard; the average IPC should not vary greater than 2Ct. If these criteria are not met, the QCO may repeat any portion of the QC testing. All criteria shall be satisfied prior to accepting the new lot of Quantifiler® Duo DNA standard for use in the Section.

5.3.2.4 **Normalization Verification:** the Standard(s) Traceable to NIST shall be averaged based upon the values obtained in **5.3.2.3** and prepared for amplification (at a minimum in triplicate) per current Section procedures. Data shall be evaluated and compared to current normalized settings. If normalization is necessary, refer to the Procedure for Calibration and Equipment Maintenance.

5.3.2.5 The kit information (lot numbers, date verified, and expiration date) shall be entered into the FA system per **5.2.1.3** by the QCO.

5.3.2.6 The general supply of Quantifiler® Duo kits shall be stored at -20 °C by the QCO; active working stock shall be kept at 4 °C or -10 °C.

5.3.3 **DNA Investigator:** Prior to use in the Forensic Biology Section, the performance of each lot of DNA Investigator kits shall be checked by the QCO against the NIST-TS as described below.

5.3.3.1 The following items shall be extracted, quantitated, amplified, electrophoresed and analyzed according to applicable Forensic Biology Section DNA Procedures:

5.3.3.1.1 Standard Traceable to NIST.

5.3.3.1.2 Negative Extraction Control (Neg K).

5.3.3.2 The Standard Traceable to NIST shall produce the expected results at all loci tested. Alleles shall be balanced within and between loci and peak heights generally between 1000 and 6000 RFUs.

5.3.3.3 The Neg K shall not exhibit any alleles.

5.3.3.4 If the kit fails to meet either **5.3.3.2** or **5.3.3.3** (for reasons other than instrument failure, known artifacts), it may be retested once. If the kit fails this second re-test, it shall not be accepted for any use in the Section and the DNA Technical Leader and kit manufacturer shall be notified immediately by the QCO.

5.3.3.5 The kit information (lot numbers, date verified, and expiration date) shall be entered into the FA system by the QCO as provided in **5.2.1.3**.

5.3.3.6 The general supply of DNA Investigator kits shall be stored at 15-25°C by the QCO.

5.3.4 Qiagen Proteinase K

5.3.4.1.1 Prior to use in the Forensic Biology Section, the performance check of Qiagen ProK shall be checked by the QCO against the NIST-TS as described below:

5.3.4.1.1.1 The following shall be extracted, quantitated, amplified, electrophoresed, and analyzed using currently quality control tested kits according to applicable Section procedures: Standard Traceable to NIST and a Negative Extraction Control (NegK).

5.3.4.1.1.2 The Standard Traceable to NIST shall produce the expected results at all loci tested.

5.3.4.1.1.3 Alleles shall be balanced within and between loci and give peak heights between 1000 and 6000 RFUs.

5.3.4.1.1.4 If the Qiagen ProK lot fails to meet either **5.3.3.7.2.2** or **5.3.3.7.2.3** (for reasons other than instrument failure or known artifacts), the lot may be retested once. If the Qiagen ProK lot fails this retest, it shall not be accepted for any use in the Section and the DNA Technical Leader and the manufacturer shall be notified immediately by the QCO.

5.3.4.1.1.5 The kit information (lot numbers, date verified, and expiration date) shall be entered into FA by the QCO as provided in **5.2.1.3**.

5.3.4.1.2 After the lot passes the performance check, aliquot 250 µl into blue-colored sterile 0.5mL tubes while under a Biological Safety Cabinet (or equivalent).

5.3.4.1.3 Aliquots shall be stored at 15-25 °C.

5.3.4.1.4 Aliquots expire 1 year after date of receipt of stock supply.

5.4 Expiration Dates for Commercial Reagents Without Manufacturer-Provided Dates

5.4.1 The following reagents shall have an expiration date set 5 years from date of receipt or preparation within the Forensic Biology Section:

- Nuclease-free dH_2O.

5.4.2 The following reagents shall have an expiration date set 3 years from date of receipt or preparation within the Forensic Biology Section:

- Dithiothreitol (stock supply).

5.4.3 The following reagents shall have an expiration date set 2 years from date of receipt or preparation within the Forensic Biology Section:

- Hi-Di Formamide (stock supply).
- LIZ sizing standard.
- 10X Buffer.

5.4.4 The following reagents shall have an expiration date set 1 year from date of receipt or preparation within the Forensic Biology Section:

- Proteinase K provided by Qiagen (aliquots).
- Proteinase K (provided in the DNA Investigator kit).
- Dithiothreitol (aliquots).
- Hi-Di Formamide (aliquots).
- 20 % SDS (in solution, purchased from an outside supplier).

- Spectral/matrix kits for 3130XL (or equivalent) and 7500 (or equivalent).
- For those reagents which are aliquoted, both the date of preparation and expiration shall be marked on the container along with reagent description, initials of preparer, and lot number (unless already covered by previously listed items).

5.4.5 The following reagents expire 1 week after preparation within the Forensic Biology Section:

- 1X buffer.

5.4.6 If the reagent container is too small for individual notation of expiration dates, it shall be noted on the parent container (box, bag, bottle or equivalent) storing the main supply of reagents. Lot numbers for reagents can also be checked against FA.

5.4.7 Reagent expiration dates shall be noted in FA by the QCO. Expired reagents shall be disposed of appropriately and not retained in the section.

6.0 Limitations - See **5.0**.

7.0 Safety

7.1 When using HCl in the preparation of Tris-HCl, extreme caution shall be used due to its corrosive nature including wearing eye protection and other personal protective equipment.

7.2 DTT, SDS: when using these chemicals in powder form, masks shall be worn due to the potential as strong respiratory irritants.

7.3 Buffer/Reagent preparation: safety glasses shall be worn at all times when preparing the STR buffers and associated reagents/solutions, unless working behind a BioSafety Cabinet/Fume hood.

7.4 Formamide is a known chemical hazard and can cause eye, skin and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Wear appropriate eyewear, gloves and clothing when in use.

8.0 References

Forensic Biology Section Procedure for Safety

Forensic Biology Section Procedure for DNA Extractions Using the EZ1 Advanced XL

Forensic Biology Section Procedure for PCR Amplification for Casework

Forensic Biology Section Procedure for Human DNA Quantitation with Quantifiler® Duo

Forensic Biology Section Procedure for Use of the 3130XL Genetic Analyzer for Casework

Forensic Biology Section Procedure for Body Fluid Unit Quality Control

Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

9.0 Records

- Temperature Charts for Freezers/Refrigerators
- QC Testing Worksheets
- Identifiler®Plus Kit QC Form
- Quantifiler® Duo Kit QC Form
- QC Testing Worksheet Templates
- DNA Investigator Kit QC Form

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document
12/7/2012	2	5.1.3 - extend lifetime for NIST-traceable standards; 5.2.1.3 - added phenol/chloroform and 10X buffer to list recorded in FA; 5.3.3 - changed QC check procedure for Quantifiler kit; 5.3.4 - removed section for Y Quant, removed references to Y-Quant in definitions; 5.2.1.3, 5.2.4.12, 5.2.4.7.7.1 - changed shall to required to
12/22/2012	3	Removed BSA – definitions, 5.2.1.2, 5.2.1.3, 5.4.2., 5.4.4; Added Identifiler Plus and Quantifiler Duo and removed Yfiler and Quantifiler Human – definitions, 5.2.1.3, 5.2.4.4.6.1, 5.2.4.5.8.1, 5.2.4.6.7.1, ; Changed 7000 to 7500 – definitions, 5.3.3, 5.4.4; Removed threshold and injection time - 5.1.2.3, 5.2.4.4.6.1, 5.2.4.5.8.1, 5.2.4.6.7.1, 5.3.1.1, 5.3.2.1; Changed "activity or peaks" to "alleles" -5.1.2.4, 5.1.2.5, 5.2.4.4.6.3, 5.2.4.5.8.3, 5.2.4.6.7.2, 5.3.1.3, 5.3.2.3 ; 5.2.4.5 – Removed Quantitation TE (DNA Standards) section; 5.2.4.6.7.1 – Removed "neat"; Removed 5.2.4.10 STR BSA Section; 5.2.4.10 – renamed quantitation standard TE to buffer; Removed 5.2.4.12.2; 5.2.4.10.3 – removed preparation requirement; 5.3.1 – removed casework unit; 5.3.2 – changed YFiler to Identifiler Plus throughout section; 5.3.2.1 – added requirement to quantitate 9947A; Changed 007 to 9947A – 5.3.2.1.2, 5.3.2.2; Removed 5.3.2.1.3; Removed 9947A – 5.3.2.3; Added requirement for 9947A concentration – 5.3.2.6; Removed requirement for Taq storage – 5.3.2.7; 5.3.3 – Changed "Human" to "Duo" throughout; Added note to QC Duo kit with no current kit – 5.3.3; 5.3.3.1 – Added requirement to only utilize human results; 5.3.3.1.3 – added "DNA"; 5.3.3.2 – corrected section reference numbers; 5.3.3.2.4, 5.3.3.3.2 – clarify comparison for Duo curves and metrics; 5.3.3.5 – removed storage requirement for reaction mix; References – updated procedures titles; Records – removed BSA and Quantifiler Human Kit

		QC forms, Changed YFiler to Identifiler Plus form
09/25/2013	4	Header – added issuing authority; 3.0 – added ProK and Carrier RNA to commercial reagent; added DNA Investigator to critical reagent; 5.1.2.2 – updated procedure name; 5.2.1.1 – added 3M NaOAc; 5.2.1.2 – added carrier RNA; 5.2.1.3 – added DNA Investigator kit, ProK and carrier RNA; 5.2.4.4.6.2 – edited to quant step; 5.2.4.10 – inserted 3M NaOAc; 5.2.4.11 – inserted Carrier RNA; 5.2.4.12.1, 5.2.4.12.3 – updated quant standards preparation; 5.3.1.1.1, 5.3.1.3 – changed Neg K to negative extraction control; 5.3.1.2 – revised to add how multiple punches are treated; 5.3.4 – inserted DNA Investigator kit; 5.4.4 – added ProK (DNA investigator kit); 8.0 – updated extraction procedure name; grammar corrections in document
12/18/2013	5	1.0, 2.0, 3.0, 5.1.1, 5.1.2.6, 5.2.1.3, 5.2.4.7.3, 5.2.4.9– removed references to DNA database Unit/reagents; 5.3.1 – removed Identifiler kit (Database use only), 5.3.2 – updated section references; 5.4.3 – removed reference to ATL buffer (Database use only); 8.0 – removed Database procedure references; Changed Units to Section throughout; reworded NIST Traceable Standard to Standard Traceable to NIST throughout
04/18/2014	6	3.0, 5.2.1.3, 5.4.3 – removed phenol/chloroform; 5.2.4.1.7, 5.2.4.2.5, 5.2.4.3.5, 5.2.4.4.5, 5.2.4.5.7, 5.2.4.6.6, 5.2.4.8.3, 5.2.4.9.5, 5.2.4.10.4, 5.2.4.10.5 – changed recording from worksheet to FA; 5.2.4.7 – removed STR-ProK section; 5.2.4.9.3, 5.2.4.11.4 – added reference for naming; 5.3.4 – added new section for QC of Qiagen ProK; 5.4.2, 7.2 – removed ProK; 5.4.4 – added Qiagen; 5.4.5 – removed (Database use only); 9.0 – removed forms now kept in FA, added DNA Investigator Kit QC Form
12/28/2015	7	5.2.4.8.1, 5.2.4.10.2 – updated aliquot amounts; 5.2.4.11.1 – clarified amounts to use in preparation; 5.3.1.7 – updated storage; 5.3.2 – updated Quant Duo kit QC procedures