Procedure for DNA Reagent Preparation and Quality Control

1.0 Purpose - To specify the required elements for the preparation of, and quality control procedures for, reagents used within the DNA Database Section.

2.0 Scope – This procedure applies to the DNA Database Forensic Scientists in the DNA Database 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 DNA Database Section shall establish the expiration date.
  Commercial reagents: ProK (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, phenol/chloroform (or equivalent), ATL buffer for Qiagen BioRobot Universal, spectral/matrix kits for 3130XL (or equivalent), Carrier RNA from the Qiagen MDx Media Kit.
  See **5.4** for specific expiration dates.
- **Critical Reagent:** Determined by empirical studies or routine practice to require reliability testing on established samples before use on database or known samples. All critical reagents shall have an expiration date as established by the manufacturer or the DNA Database Section.
  Critical reagents: STR-Tris-EDTA (STR-TE), STR-Stain Extraction Buffer (STR-SEB), commercially supplied kits and their components (AmpF*l*STR® Identifiler®)
- **QCO**: Refers to the DNA Database Quality Control Officer or designee(s).

4.0 Equipment, Materials and Reagents

- Chemicals: concentrated hydrochloric acid (HCl), sodium hydroxide pellets (NaOH), ethylenediaminetetraacetic acid (EDTA), granular (EDTA), sodium chloride, granular (NaCl), sodium dodecyl sulfide (SDS), Trizma base (Tris), glycogen
- 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
- Lab equipment to include: (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 in the DNA Database 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, 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 established previously (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 (see DNA Database Section Procedure for Organic DNA Extractions) along with an associated negative extraction control, 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 negative extraction control, as well as any extracted NIST SRMs shall then be quantitated as performed by the Forensic Biology QCO, 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 DNA Database Section DNA procedures.

5.1.2.4 The bloodstain and all NIST SRMs shall provide the expected allele calls, and all testing negatives (including negative extraction control) 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 If 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) then 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 (i.e., 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 DNA Database 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 extraction control 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

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)
- Formamide (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®)
- Kit components (e.g. reaction mix, primer, Taq, DNA standard, allelic ladder, positive and negative amplification controls)
- 3130XL and 10X buffer
- ProK, Hi-Di formamide, nuclease-free dH_2O (stock)
- SDS, HCl, EDTA, NaOH, NaCl, Tris base
- Phenol/chloroform (or equivalent)

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 DNA Database 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 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 worksheet.

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 worksheet.

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 worksheet.

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 as per worksheet.

5.2.4.4.6 QC Testing:

5.2.4.4.6.1 A sample of the NIST-TS and negative extraction control shall be extracted, 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 only at the extraction step; all subsequent steps which require the addition or use of STR-TE shall use only currently QC checked STR-TE.

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 negative extraction control shall be free of any alleles. If either condition is not met (for reasons other than instrument failure or known artifacts), then **t**he 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 **5.2.4.3.4**), 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 worksheet.

5.2.4.5.8 QC Testing:

5.2.4.5.8.1 A sample of the NIST-TS and negative extraction control shall be extracted, amplified, electrophoresed, and analyzed according to applicable DNA Database Section 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 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-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-Proteinase K (ProK)

Chemical/Reagent	Amount (for 10 mL)	Amount (for 25 mL)
Proteinase K (stock)	100 mg	0.25 g
Sterile nuclease-free dH_2O	10 mL	25 mL

5.2.4.6.1 Add water to ProK to reconstitute and mix well.

5.2.4.6.2 Aliquot 100 µL into blue-colored sterile 0.5 mL tubes while under a Biological Safety Cabinet (or equivalent).

5.2.4.6.3 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 by the DNA Database Forensic Scientist. The master supply of aliquots shall then be stored at -20 °C; working stock supplies of ProK shall be kept at -10 °C.

5.2.4.6.4 See **5.2.1.2** and **5.2.1.3** for naming convention and FA entry.

5.2.4.6.5 Aliquots expire 1 year after date of reconstitution, or when stock supply expires, whichever occurs first. Record information in worksheet.

5.2.4.7 Hi-Di Formamide

5.2.4.7.1 The QCO shall thaw formamide to 4 °C and aliquot 180 µL into autoclaved clear 1.5 mL sterile tubes; 1260 µL may be aliquoted for database if using a whole 96-well plate.

5.2.4.7.2 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 DNA Database Forensic Scientist. Aliquots expire 1 year after date of preparation, or when stock supply expires, whichever occurs first.

5.3 QC of Commercial Kits

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

5.3.1.1 The following items shall be amplified, electrophoresed and analyzed according to applicable DNA Database Section Procedures:

5.3.1.1.1 NIST_TS and associated negative extraction control (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 NIST-TS 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 negative extraction control and negative amplification controls shall not exhibit any alleles.

5.3.1.4 The allelic ladder associated with the new lot of Identifiler 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, expiration date) shall be entered into the FA system per **5.2.1.3** by the QCO.

5.3.1.7 The general supply of kits shall be stored at -20 °C by the QCO; active working stock shall be kept at 4 °C, except for Taq which shall be kept at a temperature of at least -15 °C.

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 DNA Database 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 DNA Database Section:

- Proteinase K (stock supply).

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

- Phenol/chloroform (or equivalent).
- Hi-Di Formamide (stock supply).
- LIZ sizing standard.
- 10X Buffer.
- ATL Buffer for Qiagen BioRobot Universal.

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

- Proteinase K (aliquots).
- Hi-Di Formamide (aliquots).
- 20% SDS (in solution, purchased from an outside supplier).
- Spectral/matrix kits for 3130XL (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 2 weeks after reconstitution within the DNA Database Section:

- Carrier RNA from the Qiagen MDx Media Kit.

5.4.6 The following reagents expire 1 week after preparation within the DNA Database Section:

- 1X buffer.

5.4.7 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.

5.4.8 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 ProK, SDS: when using these chemicals in powder form, masks shall be worn due to their 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

DNA Database Section Procedure for Safety and Hazardous Waste Disposal

DNA Database Section Procedure for Organic DNA Extractions

DNA Database Section Procedure for PCR Amplification with Identifiler®

DNA Database Section Procedure for Use of the 3130XL Genetic Analyzer

DNA Database Section Procedure for Aseptic Technique and Contamination Control

9.0 Records

- Temperature Charts for Freezers/Refrigerators
- 0.5 M EDTA Worksheet
- 1 M Tris-HCl Worksheet
- 20% SDS Worksheet
- STR-TE Worksheet
- STR-SEB Worksheet
- ProK Worksheet
- QC Testing Worksheets
- Identifiler® Kit QC Form
- QC Testing Worksheet Templates

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
12/18/2013	1	Original Document