Procedure for DNA Reagent Quality Control

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

2.0 Scope – This procedure applies to the Forensic Scientists in the DNA Database Section.

3.0 Definitions – See Section Definitions List

4.0 Equipment, Materials and Reagents

- 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 (or equivalent), pipettes and associated tips, cleaned and sterilized glassware, 96-well trays and septa

5.0 Procedure

5.1 NIST SRM/ Standard Traceable to NIST

5.1.1 Purpose and Use: The NIST SRM or NIST-TS shall be tested when substantial changes, new procedures, or new platforms are validated, as well as prior to use of commercially produced kits.

5.1.2 Creating a Standard Traceable to NIST:

5.1.2.1 Consecutively collect multiple buccal swabs from the donor using Bode buccal collectors. Allow the collectors to dry completely. This collection shall be considered a single batch.

5.1.2.2 A punch from a buccal swab in this batch shall then be amplified as specified in DNA Database section procedures for PCR amplification along with associated amplification controls and a NIST standard.

5.1.2.2.1 A buccal swab from the batch must be run with PowerPlex® Fusion, corresponding controls, and a NIST standard for the samples in the batch to become NIST traceable with PowerPlex® Fusion.

5.1.2.2.2 A buccal swab from the batch must be run with PowerPlex® Y23, corresponding controls, and a NIST standard for the samples in the batch to become NIST traceable with PowerPlex® Y23.

5.1.2.3 The amplified punch, controls, and NIST SRM shall then be simultaneously electrophoresed and shall be analyzed as a set according to applicable DNA Database Section procedures.

5.1.2.4 The NIST SRM shall provide the expected allele calls, and all testing negatives 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 buccal swab and NIST SRM simultaneously once. If both conditions are not met this second time, a new lot of buccal swabs 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 buccal swabs shall be accepted as a suitable NIST-TS and the entire lot of buccal swabs shall be named/referred to by the initials of the DNA donor, followed by the date on which the buccal swabs were prepared and the number in the series of collection (e.g., XXX_12012010_5). The QCO shall document the testing performed and retain this documentation, along with the NIST SRM documentation provided by the manufacturer in the Section.

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 buccal swabs from a male person. This batch may be the same NIST-TS currently in use if a sufficient quantity remains available for testing.

5.1.3 Standard Traceable to NIST: A NIST traceable standard shall be available for testing purposes. A NIST traceable standard may be used in lieu of a NIST SRM to create a new lot of NIST-traceable standard as described in the "Creating a Standard Traceable to NIST" section of the procedure. NIST traceable standards shall be appropriately stored by the QCO and shall have limited access.

5.2 Preparation and QC of Reagents/Solutions/Standards

5.2.1 Naming/Recording of Reagents/Solutions/Standards:

5.2.1.1 Formamide (aliquots) shall be recorded in FA under the Resource Manager by the QCO as follows: Item lot number_expiration date (e.g., A9815D0209_03152011).

5.2.1.2 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 (e.g., PowerPlex® PunchSolution Kit, PowerPlex® Fusion Amplification Kit, PowerPlex® Y23 Amplification Kit)
- Kit components (e.g., PunchSolution, AmpSolution, Master Mix, Primer Pair Mix, Amplification Grade Water, 2800M, Allelic Ladder, Internal Lane Standard)
- 3500xL polymer, anode buffer containers, cathode buffer containers, and conditioning reagent
- Hi-Di formamide, nuclease-free dH_2O (stock)

5.2.1.3 The serial number and lot number for the 3500xL capillary array shall be recorded on any Spatial Calibration Report and Spectral Calibration Report relating to its installation or calibration.

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 in association with the preparation or QC check of any reagents, kits, or standards shall be stored by the QCO in the QC files and thereafter retained in the Section.

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

NOTE: Glass bottles shall be cleaned with Alconox (or equivalent), rinsed with dH_2O and autoclaved prior to use (see DNA Database Section Procedure for Sample Processing Quality Control).

5.2.4.1 Hi-Di Formamide

5.2.4.1.1 The QCO shall thaw formamide to 4 °C. For PowerPlex® Fusion, the QCO shall aliquot 247 µL into autoclaved clear 1.5 mL sterile tubes; 988 µL may be aliquoted for database if using a whole 96-well plate.

5.2.4.1.2 The aliquots shall be frozen immediately at -10 °C. Once an 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 and Reagents

5.3.1 PowerPlex® PunchSolution Kits, Amplification Kits, and Components: the performance of each lot of PowerPlex® PunchSolution kit, PowerPlex® amplification kit, and individual components shall be checked by the QCO against the NIST-TS as described below prior to use in the DNA 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

5.3.1.1.2 Reagent Blank

5.3.1.1.3 2800M (positive amplification control)

5.3.1.1.4 Negative Amplification Control

5.3.1.2 Both the NIST-TS and 2800M must produce the expected results at all loci tested. Alleles must be balanced within and between loci.

5.3.1.3 The Reagent Blank and negative amplification control must not exhibit any alleles.

5.3.1.4 The allelic ladder associated with the new lot of PowerPlex® amplification kit must 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 with approval of the Technical Leader. If the kit fails this second re-test, it shall not be accepted for any use in the Section and the 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) shall be entered into the FA system by the QCO according to **5.2.1.2**.

5.3.1.7 The general supply of kits shall be stored by the QCO; Refer to DNA Database section procedures for PCR amplification for proper storage and usage of PowerPlex® PunchSolution Kit reagents and PowerPlex® amplification kit reagents.

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 2 years from date of receipt or preparation within the DNA Database Section:

- Hi-Di Formamide (stock supply).

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

- Hi-Di Formamide (aliquots).

NOTE: 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).

5.4.4 The following 3500xL reagents expire 7 days after initial use within the DNA Database Section:

- Anode buffer container
- Cathode buffer container
- POP-4

5.4.5 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 may also be checked against FA.

5.4.6 Reagent expiration dates shall be noted in FA by the QCO. Expired reagents shall be disposed of appropriately and not retained in the DNA Database Section.

6.0 Limitations - See **5.0**.

7.0 Safety

7.1 Formamide is a known chemical hazard and may cause eye, skin and respiratory tract irritation. It is a possible teratogen. Wear appropriate eyewear, gloves and clothing when in use. Refer to Appendix 1 for Chemical Hygiene and Safety Precautions."

8.0 References

DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion

DNA Database Section Procedure for PCR Amplification with PowerPlex® Y23

DNA Database Section Procedure for Safety and Hazardous Waste Disposal

DNA Database Section Procedure for Sample Processing Quality Control

DNA Database Section Procedure for Use of the 3500xL Genetic Analyzer

Laboratory Safety Manual- Chemical Hygiene Plan and Hazardous Communication Program

9.0 Records

- Temperature Charts for Freezers/Refrigerators
- QC Testing Worksheet Templates

10.0 Attachments – N/A

Revision History

Effective Date	Version Number	Reason
06/09/2023	5	5.1.1-Removed references to NIST SRM and NIST-TS

Appendix 1: Chemical Hygiene and Safety Precautions

Hi-Di Formamide (Fischer) DANGER: PARICULATLY HAZARDOUS SUBSTANCE	
	HEALTH 2 FLAMMABILITY 1 REACTIVITY 0
Detection of Release	Clear odorless liquid.
Signs/Symptoms of Exposure	May cause skin and eye irritation
PEL	NIOSH Recommended Exposure Limits (TWA) 10 ppm USA
Associated Hazards	Suspected of causing cancer. May damage fertility or the unborn child. Danger of cutaneous absorption. May cause damage to organs (Blood) through prolonged or repeated exposure if swallowed. May cause liver and kidney damage.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use tight sealing safety goggles. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time > 1 hour).
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep in a tightly closed container. Store in a cool, dry, corrosion-proof, ventilated area away from moisture, sources of heat or ignition, combustibles and oxidizers. Protect against physical damage. Dispose of in Hazardous Chemical Waste.
Emergency Procedures (2.2)(4.1)(6)	**Eye Contact:** Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Immediate medical attention is required. **Inhalation Exposure:** Remove to fresh air. If not breathing, give artificial respiration. If symptoms persist, call a physician. **Ingestion:** Never give anything by mouth to an unconscious person. Do not induce vomiting without medical advice. If swallowed, rinse mouth with water (only if the person is conscious). Risk of serious damage to the lungs (by aspiration). Get medical attention if symptoms occur. **Skin Contact:** Wash off immediately with plenty of water for at least 15 minutes. Remove and wash contaminated clothing and gloves, including the inside, before re-use. Immediate medical attention is required. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Small contained spill: wearing appropriate PPE, soak up with inert absorbent material, and place in container. Dispose in Hazardous Waste. Large spills: Evacuate area and call 911 (Haz Mat).