# 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**

- **Commercial Reagent**: A commercially produced laboratory reagent designed to conduct a specific forensic test. All commercial reagents shall have an expiration date established by the manufacturer or, if none is provided, the DNA Database Section shall establish the expiration date.
  Commercial reagents: Hi-Di formamide (both stock supply and aliquots), anode buffer container, cathode buffer container, conditioning reagent, POP-4, nuclease-free $dH_2O$, spectral/matrix kits for 3500xL (or equivalent).
- **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: Commercially supplied kits and their components (PowerPlex® PunchSolution Kit, PowerPlex® Fusion Amplification Kit)
- **QCO**: Refers to the DNA Database Quality Control Officer or designee(s).

**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 Quality Control Officer (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 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 the DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion along with associated amplification controls and a NIST SRM E (in NIST SRM 2391c).

**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. 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** Formamaide (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)
- Kit components (e.g., PunchSolution, 5X AmpSolution, 5X Master Mix, 5X Primer Pair Mix, Amplification Grade Water, 2800M, Allelic Ladder, WEN Internal Lane Standard 500)
- 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 and PowerPlex® Fusion Amplification Kits and Components:** the performance of each lot of PowerPlex® PunchSolution Kits, PowerPlex® Fusion Amplification Kits, 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® Fusion 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, 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 the Procedure for DNA Database PCR Amplification with PowerPlex® Fusion for proper storage and usage of PowerPlex® PunchSolution Kit reagents and PowerPlex® Fusion 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).
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  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.

**8.0 References**

DNA Database Section Procedure for PCR Amplification with PowerPlex® Fusion

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

**9.0 Records**

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

**10.0 Attachments** – N/A

**Revision History**

| Effective Date | Version Number | Reason |
|---|---|---|
| 08/03/2015 | 1 | Original Document |
| 04/18/2016 | 2 | 5.3 updated to reflect QC of individual components; 5.2.4.1 updated WEN volumes ; 5.4.3-removed spectral expiration date since provided by manufacturer |