Procedure for DNA Database Analysis and Technical Review of Database Samples Amplified with PowerPlex Fusion   Version 4
DNA Database Section   Effective Date: 04/18/2016
Issued by DNA Database Forensic Scientist Manager and Technical Leader

Procedure for DNA Database Analysis and Technical Review of Database Samples Amplified with PowerPlex® Fusion

1.0   Purpose – To provide a procedure for the analysis and technical review of database sample profiles generated in-house.

2.0   Scope – This procedure applies to DNA Database Section Forensic Scientists and trainees who perform DNA analysis using PowerPlex® Fusion.

3.0   Definitions – See the Procedure for DNA Database Operations for definitions applicable to this procedure.

4.0   Equipment

- Applied Biosystems GeneMapper ID-X Software

5.0   Procedure

5.1   Data Interpretation - The PowerPlex® Fusion Technical Review Sheet, the DNA Database Section Procedure for GeneMapper ID-X, and the DNA Database Section Procedure for Autosomal DNA STR Interpretation with PowerPlex® Fusion shall be used as references in data interpretation, analysis, and review.

5.2   Data Analysis

5.2.1   Analysis Methods

5.2.1.1 When samples are run manually, at least one 2800M must produce a complete profile with only the expected allele calls. If NIST-TS positive controls are run manually, at least one must produce a complete profile with only the expected allele calls.

5.2.1.2 When samples are run on the Qiagen BioRobot®, two 2800M positive controls shall be run. At least one 2800M must produce a complete profile with only the expected allele calls. If NIST-TS positive controls are run on the Qiagen BioRobot®, at least one must produce a complete profile with only the expected allele calls.

5.2.1.3 Samples shall be analyzed as listed in the DNA Database Procedure for Autosomal STR Interpretation with PowerPlex® Fusion and the DNA Database Procedure for GeneMapper® ID-X.

5.2.2   Rejected In-House Samples

5.2.2.1 For any individual samples that require reamplification or reinjection, the following steps shall be completed:

5.2.2.1.1   The sample shall be reamplified or reinjected. If samples are replated, another Forensic Scientist shall witness the process.

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5.2.2.2 For each database sample that must be reamplified or rerun, the following steps shall be completed:

    5.2.2.2.1 A sample that will not be reported with a particular GMID-X file shall be marked as "no export" in the GeneMapper ID-X project so that it is not uploaded to CODIS.

    5.2.2.2.2 If a sample needs to be rebatched, ensure a note is added to the SpecMan original batch and specimen record explaining the reason the sample was rebatched.

    5.2.2.2.3 If an analyst is not able to obtain a CODIS-eligible profile for a sample, the sample shall be rejected. Samples that exhibit potential allelic or locus dropout may be uploaded to SDIS following the CODIS procedure. A rejected sample's status shall be changed to "Rejected-Pending Return" in SpecMan. A designated DNA Database Forensic Scientist shall be notified of the rejection and the sample shall be handled as described in the Rejection Processing section of the DNA Database Sample Accessioning and Processing Procedure.

5.2.3 **Documentation for In House Runs** – Documentation for all runs (include batch number in file names) shall include:

5.2.3.1 For samples processed robotically, documentation (with file name, date, initials, page number, and lot numbers) shall include:

    5.2.3.1.1 DNA Database PowerPlex® Fusion Punch/Amplification Sheet (.pdf)

    5.2.3.1.2 Qiagen Amplification Report File (.pdf)

5.2.3.2 For samples processed manually, documentation (with file name, date, initials, page number, and lot numbers) shall include:

    5.2.3.2.1 Punch/Amplification setup sheet (.pdf)

5.2.3.3 For all samples analyzed (robotically and manually), documentation (with file name, date, initials, page number, and lot numbers) shall include:

    5.2.3.3.1 Scanned (or filled in electronically as PDF), completed lab worksheets from either the worksheets listed in sections **5.2.3.1.1** through **5.2.3.1.2** (for robot) or listed in **5.2.3.2.1** (for manual) and any additional handwritten bench notes, if applicable.

    5.2.3.3.2 3500xL tray setup sheet (.pdf).

    5.2.3.3.3 Raw data folders.

    5.2.3.3.4 Edited GeneMapper ID-X file (.ser), project file as reported including edits (file provides access to review electropherograms, negative controls, and WEN ILS 500).

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5.2.3.3.5   Casework table (.pdf) - includes all samples run in file (required for CODIS hit confirmations only).

5.2.3.3.6   Allele call table (required for CODIS hit confirmations only).

5.2.3.3.7   Exceptions/Notes (.pdf).

5.2.3.3.8   CMF file (.xml) generated for all samples to be uploaded/updated. The CMF file shall be deleted after successful upload of all samples to CODIS.

    5.2.3.3.8.1   The specimen category for each sample shall be appropriately identified as "Convicted Offender", "Offender-State", "Arrestee" or "Arrestee-State."

    5.2.3.3.8.2   This file is generated from GeneMapper ID-X as a CMF 3.2. Both the source and destination CODIS laboratory IDs shall be set to NCBCI0094.

5.2.3.3.9   All unused data including raw data folders, GeneMapper ID-X files, and casework tables for each project.

5.2.4   The DNA Database Forensic Scientist shall complete the following:

5.2.4.1   Compare the allele call table (if present) to the electropherograms for concordance.

5.2.4.2   Review the samples for eligibility into CODIS.

5.2.4.3   Examine the CMF file to ensure that only the sample numbers reported are present in the file.

5.2.5   Once analysis has been completed, the DNA Database Forensic Scientist shall change the batch status to "Analysis Complete – Pending Review" in SpecMan and assign the batch to a reviewer.

NOTE: The Forensic Scientist Manager and Technical Leader shall be notified in the event of interpretation or technical issues. The Technical Leader shall determine the appropriate course of action.

5.3   **Technical Review of In-House Data Analysis**

5.3.1   A second, qualified DNA Database Forensic Scientist shall review all samples for quality as described in the Documentation for In-House Runs section above and as indicated on the PowerPlex® Fusion Technical Review Sheet. The technical review of data shall consist (at a minimum) of the following:

5.3.1.1   A review of all notes, all documentation, and all electronic data (used and unused).

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5.3.1.2 A review of all controls, internal lane standards, and allelic ladders to verify that the scientifically expected results were obtained.

5.3.1.3 A review of any reworked samples to confirm that the samples have the required controls.

5.3.1.4 A review of all profiles to verify that they are supported by the raw or analyzed data.

5.3.1.5 A review for sample entry eligibility into CODIS. A review of the CMF file to ensure that the specimen category is correct and that only the sample numbers reported are present in the file.

5.3.2 A PowerPlex® Fusion Technical Review Sheet shall be completed as a part of every technical review.

5.3.3 If the reviewer determines any corrections are needed, the corrections shall be listed in the Reviewer Notes section of the PowerPlex® Fusion Technical Review Sheet. The original DNA Database Forensic Scientist shall make corrections and then initial and date the Reviewer Notes section. The reviewer shall then review the updated items for accuracy and complete the PowerPlex® Fusion Technical Review Sheet.

5.3.4 If the reviewer determines that a sample is not acceptable during the review process, the following steps shall be completed:

5.3.4.1 The reviewer notes the specimen number and reason for rejection on the PowerPlex® Fusion Technical Review Sheet. Samples shall be reprocessed as outlined in **5.2.2.2**.

5.3.4.2 The reviewer ensures the original DNA Database Forensic Scientist corrects the allele call table, CMF file (if required), and the edited GeneMapper ID-X file. Corrections and modifications shall be made in accordance with **5.3.3**.

5.3.4.3 The reviewer then reviews the updated items listed in **5.3.4.2** for accuracy.

5.3.5 Technical Issues: If during the course of a review, the reviewer and DNA Database Forensic Scientist are unable to resolve a technical issue, the Technical Leader shall be notified of the issue by the DNA Database Forensic Scientist. The Technical Leader shall then determine and/or approve the appropriate course of action.

5.3.6 Once the review has been completed, the reviewer shall change the batch status to "Review Complete" in SpecMan signifying that the above criteria has been completed and that the allele call table (if present) is concordant with the electropherograms and CMF (if present). The reviewer shall reassign the batch to the original DNA Database Forensic Scientist.

5.3.7 After the data has been properly saved, the DNA Database Forensic Scientist shall set the batch status to "Reviewed – Pending CODIS Upload" in SpecMan for all batches to be uploaded to CODIS. For batches (e.g., confirmation) that are not to be uploaded to

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CODIS, refer to the Documentation for In-House Runs section of this procedure and the appropriate section of the Procedure for DNA Database Operations.

5.4   Upload

5.4.1   Once the manifest status has been changed to "Reviewed – Pending CODIS Upload" as provided in DNA Database procedures, the CMF file shall be uploaded following CODIS Procedures.

5.4.2   Review the SDIS Import Reconciliation Report. If it indicates any problems, the DNA Database Forensic Scientist shall ensure all problems are resolved according to NDIS requirements.

5.4.3   The DNA Database Forensic Scientist shall add CODIS upload date and change the batch status to "Stored – Entered in CODIS" in SpecMan.

5.4.4   The PowerPlex® Fusion Technical Review Sheet shall be dated and initialed by the DNA Database Forensic Scientist indicating upload and specimen manager system update were completed.

5.4.5   The Forensic Scientist Manager or a qualified DNA Database Forensic Scientist shall indicate verification of the allele calls in CODIS by noting such and initialing on the PowerPlex® Fusion Technical Review Sheet if the calls are entered manually by a DNA Database Forensic Scientist.

5.5   Documentation

5.5.1   The technical review sheet generated for the batch shall be saved to the server in a non-editable format.

5.5.2   The electronic data containing all files associated with the batch shall be saved to the server.

5.5.3   All analysis documentation and technical review sheets shall be retained.

6.0   Limitations – N/A

7.0   Safety – N/A

8.0   References

DNA Database Section Administrative Policy and Procedure

DNA Database Section Administrative Procedure for Safety and Hazardous Waste Disposal

DNA Database Section Procedure for Autosomal DNA STR Interpretation with PowerPlex® Fusion

DNA Database Section Procedure for DNA Database Operations

DNA Database Section Procedure for DNA Database Training for PowerPlex® Fusion

DNA Database Section Procedure for GeneMapper ID-X

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DNA Database Section Procedure for Qiagen BioRobot® Universal Using PowerPlex® Fusion

Procedure for CODIS

State Crime Laboratory Safety Manual

9.0   Records

- BioRobot® DB Runs Notebooks
- Electronic Documentation
- PowerPlex® Fusion Technical Review Sheet

10.0   Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
08/03/2015	1	Original Document
10/09/2015	2	5.2.3.3.6 - removed PDF requirement for allele call tables from in-house analysis
12/21/2015	3	5.2.3.3.8 – update .dat to .xml and include update profile ; 5.2.2.2.2 removed note in specimen record;5.2.2.2.3 added upload SDIS for partial sample; 5.2.3.3.5 clarified casework table for CODIS hit confirmations; 5.2.3.3.8.2 remove entire CMF NOTE; 5.2.3.3.8.1 Added SDIS levels; 5.5.1 tech review sheet not printed
04/18/2016	4	5.2.2.2.2 updated batching note requirement; 5.2.3.3.8- CMF file deleted after upload to CODIS