
Procedure for DNA Database Analysis and Technical Review of Database Samples

- 1.0 Purpose** –To provide a procedure for the analysis and technical review of quality control samples processed in-house, CODIS hit confirmations, and database sample profiles generated in-house prior to upload into CODIS.
- 2.0 Scope** – The procedures in this document apply to the DNA Database Section of the State Crime Laboratory. Qualified DNA Database Forensic Scientists who have completed the DNA Database Training Program shall complete the responsibilities outlined herein.
- 3.0 Definitions** – See the DNA Database Section Procedure for definitions applicable to this procedure.
- 4.0 Equipment**
- Applied Biosystems GeneMapper ID® Software
- 5.0 Procedure**
- 5.1 Data Interpretation** - The In-House Technical Review Sheet, the DNA Database Section Procedure for GeneMapper ID and DNA Database Section Procedure for Autosomal DNA STR Interpretation shall be used as references in data interpretation, analysis, and review.
- 5.2 Data Analysis**
- 5.2.1 Analysis Methods**
- 5.2.1.1** The “NCSBI Robot” analysis method (found on Section shared drive) shall be used to analyze database samples run on the Qiagen BioRobot®. Samples shall be analyzed at a threshold value between 100 and 175 RFUs.
- 5.2.1.2** When analyzing data generated from the Qiagen BioRobot®, if positive extraction controls are processed, at least one of the three known extracted positive controls must produce a complete profile with the expected allele calls. At least two of the three positive extraction controls shall be devoid of extraneous alleles.
- 5.2.1.3** The “G5_Advanced” analysis method (found on Section shared drive) shall be used to analyze database samples extracted manually. Samples shall be analyzed using a 100 RFU threshold.
- 5.2.2 Rejected Samples**
- 5.2.2.1** For any individual sample(s) that require re-amplification or reinjection, the following steps shall be completed:
- 5.2.2.1.1** The sample shall be re-amplified or re-injected and remain part of its original batch unless approved by the Forensic Scientist Manager (e.g., another DNA Database Forensic Scientist must perform the re-amplification or reinjection.) In the event that a new batch is created for a sample, the applicable steps in 5.2.2.2 shall be completed.

- 5.2.2.1.2 If the sample being re-amplified or re-injected is part of a 96-well extraction or amplification tray, the well numbers from which the sample is taken and the sample is dispensed shall be recorded. This process shall be witnessed by another qualified DNA Database Forensic Scientist. The witness shall record the date and his/her initials on the worksheet.
 - 5.2.2.2 For each database sample that must be rejected and rerun (e.g., re-extracted or no profile obtained), the following steps shall be completed:
 - 5.2.2.2.1 The sample shall be marked as “no export” in the Edited GeneMapper ID project so that it is not uploaded to CODIS.
 - 5.2.2.2.2 A note shall be added to the specimen record in SpecMan describing, in detail, the reason for rejection.
 - 5.2.2.2.3 The specimen status shall be changed to “Rejected – Reprocess.”
 - 5.2.2.2.4 The designated DNA Database Forensic Scientist shall be notified that the specimen requires reanalysis.
- 5.2.3 If the sample has an associated QC sample and the profiles are not concordant, the Forensic Scientist Manager shall be notified. The Forensic Scientist Manager shall determine the root cause.
 - 5.2.3.1 If the root cause is a vendor problem, the samples shall not be uploaded to CODIS. The manifest status shall be changed to “Failed QC.” The non-QC batches in the manifest shall be changed to “Stored – Pending Analysis.” A note shall be added to the manifest record describing the issue.
 - 5.2.3.2 If the root cause is not a vendor problem and is able to be resolved, a note shall be added to the specimen record and the review process shall continue.
- 5.2.4 Documentation for all runs (include batch number in file names) shall include:
 - 5.2.4.1 For samples extracted robotically, documentation (with file name, date, initials, page number, and lot numbers) shall include:
 - 5.2.4.1.1 NC State Crime Laboratory DNA Database Identifiler Punch/Extraction Sheet (.pdf)
 - 5.2.4.1.2 Qiagen Extraction Report File (.pdf)
 - 5.2.4.1.3 NC State Crime Laboratory DNA Database Identifiler Amplification Sheet (.pdf)
 - 5.2.4.1.4 Qiagen Amplification Report File (.pdf)
 - 5.2.4.2 For samples extracted manually, documentation (with file name, date, initials, page number, and lot numbers) shall include:
 - 5.2.4.2.1 Extraction Worksheet (.pdf)

5.2.4.2.2 Amplification Worksheet (.pdf)

5.2.4.3 For all samples analyzed (robotically and manually), documentation shall include:

5.2.4.3.1 Scanned, completed lab worksheets from either the worksheets listed in sections **5.2.4.1.1** through **5.2.4.1.4** (for robot) or listed in **5.2.4.2.1** and **5.2.4.2.2** (for manual) and any additional handwritten bench notes, if applicable.

5.2.4.3.2 3100/3130 Tray Setup Sheet (.pdf)

5.2.4.3.3 Raw data folders

5.2.4.3.4 Edited GeneMapper ID file (.ser), project file as reported including edits

5.2.4.3.5 Negative control primer peaks documentation (.pdf)

5.2.4.3.6 Electropherograms (.pdf)

5.2.4.3.7 LIZ sizing standard as separate set of electropherograms (.pdf)

5.2.4.3.8 Casework Table (.pdf), includes all samples run in file

5.2.4.3.9 Allele call table (.pdf), found on section shared drive

5.2.4.3.10 Exceptions/Notes (.pdf), notes samples run but not reported

5.2.4.3.11 CMF file (.dat), generated for all samples to be uploaded

5.2.4.3.11.1 The specimen category for each sample shall be appropriately identified as “Convicted Offender” or “Arrestee.”

5.2.4.3.11.2 This file is generated from GeneMapper ID as a CMF1.0. Both the source and destination CODIS laboratory IDs shall be set to NCBCI0094.

Note: QC samples that are run in-house (generated for a manifest sent to an outsourcing vendor) and confirmation samples shall not be uploaded to CODIS, therefore, a CMF file shall not be generated for these samples.

5.2.4.3.12 All unused data including raw data folders, GeneMapper ID files, electropherograms, LIZ sizing standard electropherograms, negative control primer peak documentation, and casework tables for each project.

5.2.5 The DNA Database Forensic Scientist shall complete the following:

5.2.5.1 Compare the allele call table to the electropherograms for concordance.

5.2.5.2 Review the samples for eligibility into CODIS.

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

5.2.6 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 Data Analysis

5.3.1 A second, qualified DNA Database Forensic Scientist shall review all samples for quality as described above and as indicated on the In-House 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).

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 DNA types 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 An In-House Technical Review Sheet shall be completed as a part of every technical review.

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

5.3.3.1 The reviewer notes the specimen number and reason for rejection on the In-House Technical Review Sheet. The specimen shall be reprocessed as outlined in 5.2.2.2.2 through 5.2.2.2.4 and be assigned to a new batch.

5.3.3.2 The reviewer ensures the original DNA Database Forensic Scientist corrects the allele call table, CMF file (if required), and the edited GeneMapper file. The original DNA Database Forensic Scientist shall note these modifications on the Exceptions/Notes sheet of the file.

5.3.3.3 The reviewer then reviews the updated items listed in 5.3.3.2 for accuracy.

5.3.4 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 is concordant with the electronic electropherograms. If this is a QC batch, the status shall be changed to “Reviewed – Ready to Use in QC.” The reviewer shall assign the batch back to the original DNA Database Forensic Scientist.

5.3.5 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 that shall be uploaded to CODIS. For batches (i.e., QC, confirmation) that are not to be uploaded to CODIS, refer to the Documentation section of this procedure and the appropriate section of DNA Database Section Procedure.

5.4 Upload

5.4.1 Once the manifest status has been changed to “Reviewed – Pending CODIS Upload” per the 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 In-House 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 In-House 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 scanned and saved to the CODIS server.

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

State Crime Laboratory Safety Manual

DNA Database Section Administrative Policy and Procedure

DNA Database Section Administrative Procedure for Safety and Hazardous Waste Disposal

DNA Database Section Procedure

DNA Database Section Procedure for DNA Database Training

DNA Database Section Procedure for Qiagen BioRobot® Universal

DNA Database Section Procedure for GeneMapper ID

DNA Database Section Procedure for Autosomal DNA STR Interpretation

Procedure for CODIS

9.0 Records

- BioRobot® DB Runs Notebooks
- Electronic Documentation
- In-House Technical Review Sheet

10.0 Attachments – N/A

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