Inter-Laboratory Variation in Interpretation of DNA Mixtures Study

DNAmix 2021 Instructions — Registration and Questionnaires

Contents

1 Overview

This is a large-scale independent study being conducted to evaluate the extent of consistency and variation among forensic laboratories in interpretations, comparisons, and statistical analyses of DNA mixtures, and to assess the effects of numerous potential sources of variability. The study will evaluate the current state of the practice of DNA mixture casework and will not be restricted to specific products or statistical approaches. Noblis, Inc. and Bode Technology are conducting this study under National Institute of Justice (NIJ) grant award # 2020-R2-CX-0049, for the period 01/01/2021 to 12/31/2022.

This study will be composed of four phases:

1) *Policies and Procedures (P&P) Questionnaire* — Online questionnaire to assess laboratory policies and procedures relevant to DNA mixture interpretation (notably systems, types of statistics reported, and parameter settings used).

2) *Casework Scenario Questionnaire* — Online questionnaire to assess analysis procedures or decisions that may vary depending upon the case scenario and the nature of mixture casework conducted by the laboratory.

3) *Number of Contributors (NoC) Subtest* — Assessment of suitability and number of contributors for 14 mixture DNA samples, given electropherogram data.

4) *Interpretation, Comparison, and Statistical Analysis (ICSA) Subtest* — Interpretations, comparisons, and statistical analyses for 7 mixture DNA samples, given electropherogram data and reference profiles of assumed and potential contributors.

The DNAmix 2021 website (https://dnamix.edgeaws.noblis.org) will provide access to all online questionnaires and subtest electropherogram data (available for download) as well as a user interface for reporting responses for each phase of this study. This document provides instructions for registration and the first two phases (the *P&P Questionnaire* and *Casework Scenario Questionnaire*). A separate document will provide instructions for the *NoC* and *ICSA Subtests*.

2 Eligibility

Participation is open to all forensic laboratories that conduct DNA mixture interpretation as part of their standard operating procedures (SOPs). Non-U.S. laboratories are welcome to participate if they report the results of their interpretations, comparisons, and statistical analyses in English.

Participation in this study requires the participants to agree to use the same diligence in performing these analyses as used in operational casework, and to use their laboratory's SOPs in performing these analyses and conducting any quality assurance procedures required for the *NoC* and *ICSA* subtests.

Laboratories are encouraged to participate in the early phases even if they cannot commit to the later phases.

3 Registration

For the purposes of this study, participants are laboratories, not individuals. It is the discretion of participating laboratories to determine which analysts will be involved in the study, and the identities of the specific analysts will not be known to the DNAmix Study Team. Analysts involved must be qualified by the laboratory for operational mixture casework (not trainees). Laboratories will be permitted to register more than one participant, with each completing a separate registration; for additional details, please see *Section 3.1*.

Please go to the DNAmix 2021 study website (https://dnamix.edgeaws.noblis.org) to register for this study. The website is accessible using an ordinary web browser; there is no need to download or install any additional software or plugins. The website is compatible with the most recent releases of Google Chrome, Mozilla Firefox, Microsoft Edge, and Apple Safari. Using older versions may result in errors. Internet Explorer is not supported.

To register for this study, eligible laboratories must complete the following:

- Online consent form— see *Section 3.2*
- Online registration form— see *Section 3.3*
- Online configuration questionnaire— see *Section 3.4*

After completion of these items, you will use the email address that you provided along with the password you create during the registration process in order to access the DNAmix 2021 website. To enable the necessary security given that we are dealing with DNA samples, the study website requires two-factor authentication via text message to log in.

3.1 Registering multiple participants from a laboratory

Laboratories will be permitted to register more than one participant. Should a laboratory elect to submit multiple responses, each registered participant will be referred to hereafter as a "subunit." It is the laboratory's discretion whether to enroll subunits, and the particular analysts that comprise each subunit—the identities of specific individuals within a subunit will not be known to the DNAmix Study Team.

Each subunit must complete the entire registration process, including the consent form, registration form, and configurations questionnaire.

Each subunit will also be required to complete each phase of the study (the *Policies and Procedures questionnaire, Casework Scenario questionnaire*, *NoC subtest*, and *ICSA subtest)* **completely independently** from any other subunits within your laboratory or other laboratories.

If feasible, technical reviews and quality assurance procedures as outlined in the laboratory's SOPs should also be conducted for each subunit independently.

3.2 Online informed consent form

Participation in this study requires completion of an electronic informed consent form by a laboratory or subunit representative. The DNAmix 2021 website will provide access to the online consent form as part of the registration process. Please complete the required informed consent fields on the website. After completion of this form, we recommend that you print or save a PDF copy for your records.

The text for the online informed consent form is duplicated in this document in Appendix A.

3.3 Online registration form

You will then be prompted to complete the online registration form, which collects information about your laboratory and contact details for an administrative point of contact for your laboratory or subunit. Note that the cell phone number provided for the point of contact will be the one used for two-factor authentication when logging into the study website.

The text for the electronic registration form is duplicated in this document in Appendix B.

3.4 Online configuration questionnaire

The DNAmix 2021 website will then prompt you to complete the *Configuration Questionnaire*, which includes questions about the amplification kit(s) and capillary electrophoresis (CE) instrument(s) that your laboratory uses for DNA mixture casework.

Responses to the *Configuration Questionnaire* will be used by the study team in generating electropherograms for the *NoC subtest* and *ICSA subtest*. For each mixture sample and reference sample used in these subtests, we will prepare HID files for several popular combinations of "Amp/CE Settings", which refers to a specific combination of:

- Amplification kit
- Amplification cycles
- Volume of amplification reaction
- CE instrument
- Injection time and voltage

The text for the online configuration questionnaire is duplicated in this document in Appendix C.

Note that in the Configuration Questionnaire, each question is submitted individually, and cannot be revised after submission.

4 Study Questionnaires (Phases 1 & 2)

Please review the DNAmix 2021 Glossary on the DNAmix 2021 website prior to beginning the *P&P Questionnaire* and the *Casework Scenarios Questionnaire* for details about the acronyms and terminology as specifically used in this study.

Both the *P&P Questionnaire* and the *Casework Scenarios Questionnaire* automatically save your progress as you complete each set of questions, and allow you to go back a revise your responses up until the point you submit at the end of each questionnaire.

The questionnaires do not need to be answered all at one time, but can be completed over time — IF you use the same browser and computer. NOTE: if you change computers or browsers the questionnaires will restart from the beginning.

4.1 Policies and Procedures Questionnaire

The *Policies and Procedures (P&P) Questionnaire* is the first phase of this study, and will be accessible via a link on the Participant Homepage of the DNAmix 2021 website. When the *P&P Questionnaire* initially becomes available, all registered participants will be notified via e-mail.

The *P&P Questionnaire* includes questions regarding your laboratory's:

- DNA workflow (quantification, amplification, capillary electrophoresis) details
- STR analysis software specifications
- Approach for assessing number of contributors (NoC)
- Criteria used for separating major and minor contributors
- Suitability assessments
- Non-statistical conclusions reported
- Statistical analyses conducted and reported
- Conditioning considerations
- Population databases utilized
- Probabilistic genotyping software policies (if applicable)
- Non-probabilistic genotyping software policies (if applicable)
- Reporting language used

Please answer all questions as completely and accurately as possible, based upon your laboratory's SOPs and any other policies and validated procedures utilized for DNA mixture casework.

The responses from this survey will be used by the study team to assess whether differences in SOPs across laboratories explain differences in interpretations, comparisons, or statistical analysis of DNA mixtures. In addition, the responses will be used to inform the design of and data selection for the subsequent phases of this study.

4.2 Casework Scenario Questionnaire

The *Casework Scenario Questionnaire* is the second phase of this study, and will be accessible via a link on the Participant Homepage of the DNAmix 2021 website. When the *Casework Scenario Questionnaire* initially becomes available, all registered participants will be notified via e-mail.

The *Casework Scenario Questionnaire* includes questions regarding:

- Availability of a variety of case information
- Analysis options that vary at the case level
- Nature of your mixture casework

The responses from this survey will be used by the study team to assess analysis procedures or decisions that may vary depending upon the case scenario and the nature of your mixture casework.

5 Anonymity and retrieving laboratory results

5.1 Anonymity

Study records and test results will be confidential. Laboratory and subunit information will be limited to what is necessary for administering the study. Reported results will not be linked to laboratories' identifying information: results will be reported using anonymized identifiers, without attribution to specific agencies. No reference will be made in oral or written reports, publications, or released datasets that could link your laboratory's name or

contact information to the study. Reported results will not be aggregated in a way that compromises confidentiality.

Responses will be kept separate from laboratory names and contact information during analyses of results. Confidentiality will be assured through multiple levels of data anonymization, data segregation, and controlled flow of information. Cross-references between laboratory information and the anonymized identifiers will be destroyed prior to the publication or public presentation of the results. Therefore, the identities of participating agencies will not be associated with the results at any point during analysis, and such association will not be possible subsequently, such as for discovery or FOIA requests.

5.2 Retrieving laboratory results

Results will be coded in a way that will allow participants to see their own anonymized results after completion of the study, if they choose to do so. After the submission of all samples (at the end of the *ICSA Subtest*), the software will give each participant the option to see its own Anonymous ID. This Anonymous ID will only be provided once: if a participant loses its Anonymous ID after it has been provided, that participant will not be able to see its results. The software will not record whether participants accessed their Anonymous IDs. If a laboratory includes multiple participating subunits, Anonymous IDs will be provided independently to each participating subunit: subunits within a laboratory will not be provided the Anonymous IDs for the other subunits. When the testing period is complete, but before public release of results, point-of-contact information will be deleted and the cross-references between laboratory information and anonymized identifiers will be deleted: after that point the study team will have no way to associate results with individual laboratories or look up/reassign anonymized identifiers. The final report will include an appendix with a table showing the results for each anonymized identifier, allowing those laboratories or subunits who chose to keep their anonymized identifiers to look up their results.

In some legal systems, knowledge of test results may create an obligation for the laboratory to disclose the test results in criminal, civil or regulatory proceedings. Prior to requesting Anonymous IDs, participating laboratories may wish to consult with their agency's counsel.

Appendix A Informed Consent Form

On the *DNAmix 2021 website*, select "Register" and as part of the registration process you will be presented with the following informed consent form. Note that informed consent is completed online; this information is provided here as a reference.

Study Title

Inter-laboratory Variation in Interpretation of DNA Mixtures ("DNAmix 2021")

Sponsors

Noblis, Inc. and Bode Technology

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Purpose

This study will be a large-scale, independent, rigorous empirical evaluation of the extent of variation among forensic laboratories in the statistical analysis and interpretation of electropherograms (EPGs) resulting from DNA mixtures. We plan to have between 50 and 150 laboratories taking part in this study. This study is being conducted under a grant from the National Institute of Justice (NIJ Grant #2020-R2-CX-0049).

Participation

Participation will be open to all forensic laboratories that conduct DNA mixture interpretation as part of their SOPs; non-U.S. laboratories are welcome to participate if they report interpretations in English. Participation in the study requires the participants to agree to use the same diligence in performing these analyses as used operationally in casework, and to use their laboratory's SOPs in performing these analyses.

Procedures

The study will consist of four subtests:

1. Policies and Procedures Questionnaire — Online questionnaire to assess laboratory policies and standard operating procedures (SOPs) relevant to DNA mixture interpretation, interpretation or statistical software used, and parameter settings.

2. Scenario Questionnaire — Online questionnaire presenting a number of casework-derived scenarios (without DNA data), asking participants to assess how they would conduct analysis for each scenario.

3. Number of Contributors Subtest (NoC) — Assessment of suitability and number of contributors, given electropherogram data from DNA mixtures.

4. Statistical Analysis and Interpretation Subtest — Report of statistical results and categorical interpretations, given electropherogram data from DNA mixtures provided with DNA profiles of reference samples.

All of the subtests will be administered online. Participants are encouraged to participate in the early subtests even if they do not participate in the later subtests.

If your laboratory expresses interest or agrees to participate, it will be sent a link to a website that will collect the laboratory name, point of contact, and email address for the point of contact. After this consent form is submitted, a link will be sent to the Policies and Procedures Questionnaire. Once a subtest of the study is completed, a link to the next subtest in the above list will be sent.

Confidentiality

Results will be confidential. No information about your laboratory will be released. No personally identifiable information (PII) will be released and results will not be attributed to participants. The research results will be published, but anonymity of participants will be maintained and results will not be associated with specific participants. Personally identifiable information (PII) will be used only for the purpose of conducting the study, and will not be used or released for other purposes. Your laboratory's study results will not be linked to its PII. No reference will be made in oral or written reports, publications, or released datasets that could link your laboratory's name to the study. A blind coding system will ensure anonymity. The subject ID numbers associated with your laboratory will be anonymized so that the analysis team will not be able to associate your laboratory's responses to any/all of the four subtests with your laboratory's name, email address, or laboratory representative. Cross-references between the subject IDs and the anonymized codes will be destroyed prior to the publication or public presentation of any results. Therefore, the identities of participating laboratories will not be associated with the results at any point during analysis, and such association will not be possible subsequently, such as for discovery.

Upon publication of the study results, your laboratory will be offered an opportunity to see its results using an Anonymous ID that will be provided when the last responses are submitted. This Anonymous ID will only be provided once: if your laboratory loses its Anonymous ID after it has been provided, your laboratory will not be able to see its results as the researchers will have no way of linking the Anonymous ID to your laboratory's name or contact information once the study is completed. The study team will have no way of knowing if your laboratory accessed its Anonymous ID. If your laboratory chooses to obtain its results, it is solely up to your laboratory's discretion as to whether or not to share its results with anyone, except as required by law.

The researchers will not disclose which laboratories did or did not take the test. In reporting results, results will be aggregated across multiple laboratories. Care will be taken so that the results are not aggregated in a way that compromises anonymity. The Principal Investigators and the Institutional Review Board (IRB) will be able to inspect confidential study-related records that identify your laboratory by name, which means that absolute confidentiality cannot be guaranteed.

Benefits

This study is for research purposes only. There is no direct benefit to your laboratory from participation in the study. The results of this study will be published in a peer-reviewed journal. The results of this research will provide the DNA analysis community with information regarding the accuracy, reproducibility, and repeatability of analyses produced in the discipline. This information can be used to improve analysis methodologies, training programs, and quality assurance measures. If the study indicates high performance by DNA analysis laboratories, this research may provide confidence in the legal community that DNA mixture analysis is reliable and provides added value to investigations and during courtroom proceedings. This research will inform future DNA mixture analysis studies.

Risks and Discomforts

No deception will be used in this study.

Analysts may experience physical fatigue (including eye strain) and mental fatigue if they perform analyses for an extended period of time. The scenarios in the Scenario Subtest are designed to resemble real casework and may be disturbing to some people.

In some legal systems, knowledge of your laboratory's test results may create an obligation for your laboratory to disclose those results in a criminal, civil or regulatory proceeding for which your laboratory is called to testify or provide evidence. If your laboratory chooses to access its own results, that information may then be under legal discovery when the laboratory acts as an expert witness in the future. If your laboratory elects to request its individual results, your laboratory is advised to consider first consulting with your laboratory's agency's counsel or counsel of your laboratory's choice.

The study team is not aware of any other risks, but there may be unknown risks associated with this study.

New Findings

Any new important information that is discovered during the study and which may influence your laboratory's willingness to continue participation in the study will be provided to your laboratory.

Alternatives

This research study is for research purposes only. Your laboratory is free to participate or not participate in this study. If your laboratory chooses not to participate, there will be no negative consequences.

Costs and Compensation for Participation

No charges will be billed to your laboratory or your agency for this study. Your laboratory will not be paid for its participation in this study.

Whom to Contact

If your laboratory has questions about the study, please contact the study staff listed on page one of this document. Please reference "DNAmix 2021" when contacting the Principal Investigators or study staff.

Refusal or Withdrawal of Participation

Participation in this study is voluntary. Participation in this research study is not mandatory; your laboratory may withdraw from the study for any reason without penalty. If your laboratory decides to participate, completion of the entire study is encouraged, but is not required. If your laboratory withdraws from the study before data collection is completed, you may notify the principal investigators if you wish for your laboratory's data to be destroyed. After the end of the data collection period (after the results from all participants have been collected), data will be anonymized and pooled and withdrawal of your laboratory's data will not be possible. Your laboratory's decision whether or not to participate in the study will not affect its current or future relations with the investigators. The investigators or the sponsor can stop your laboratory's participation at any time without your laboratory's consent.

Injury Statement

If anyone at your laboratory becomes ill or is injured while your laboratory is in the study, that individual is encouraged to get the medical care that s/he needs right away. If anyone at your laboratory is injured while engaged in the study or as a direct result of this study, your laboratory should contact the principal investigator at the number(s) provided on the first page of this form. Your laboratory will not lose any of its legal rights or release the sponsor, the Investigator, the study staff, or study site from liability for mistakes by signing this consent document.

Data Use Agreement

Due to human subjects research restrictions, the DNA profiles and mixtures included in this study shall not be used for any purpose other than this study: they shall not be stored, retained, or shared with anyone outside your laboratory; they shall not be used for research or internal validation studies; any copies or representations of the DNA profiles and mixtures shall be destroyed at the end of the study.

Consent to Take Part in this Research Study

I confirm that the purpose of the research, the study procedures and the possible risks and discomforts as well as potential benefits that I may experience have been explained to me. Alternatives to my laboratory's participation in the study also have been discussed. All of my questions have been answered. I have read this consent form. By agreeing to this informed consent form, my laboratory agrees that it will not save, copy, or redistribute any of the files included in the study.

By selecting "I consent to taking part in this study" below, as an authorized representative of my laboratory, I freely give consent for my laboratory to take part in this study.

Appendix B Registration Questions

On the *DNAmix 2021 website*, as part of the registration process you will be asked for the following information after the informed consent form. Note that registration is completed online; this information is provided here as a reference.

Please read "DNAmix 2021 — Overview and Registration Instructions" (available on the DNAmix 2021 website) prior to completing this form. That document provides an overview of the study and details on eligibility, registration, and anonymity of results.

Please provide an administrative point of contact for this participating laboratory. Alternatively, if you are participating as part of a subunit, please provide the point of contact for your subunit. This information will only be used to administer the study. This email address will be used to log into the study website to access data and provide responses. This cell phone number will be the one used for two-factor authentication when logging into the study website: to log into the study website, a code will be texted to this cell phone that must be entered in the website to proceed.

- *Point of Contact First Name*
- *Point of Contact Last Name*
- *Point of Contact Email Address*
- *Point of Contact Cell Phone Number (Must be able to receive text messages. Do not include dashes or spaces. US phone numbers must start with "+1"; non-US numbers start with "+" and country code.)*
- *Password (Must be at least 12 characters, and include at least one each of [uppercase letters, lowercase letters, digits, and symbols])*
- *Confirm Password*

Appendix C Configuration Questionnaire

On the *DNAmix 2021 website*, as part of the registration process you will be asked for the following information after the registration form. Note that registration is completed online; this information is provided here as a reference.

Note that in the Configuration Questionnaire, each question is submitted individually, and cannot be revised after submission.

The purpose of these questions is to gather information from participating laboratories about the type of laboratory, and about the amplification kit(s) and capillary electrophoresis (CE) instrument(s) that your laboratory uses for DNA mixture casework. The study team will use this information to select the settings used in creating mixtures and resulting electropherograms that will be used in the later phases of this study (NoC subtest and ICSA subtest). For each mixture, we will create electropherograms/.HID files to accommodate as many participants as possible.

When filling out this questionnaire, please respond only regarding STR identity testing (not mitochondrial, paternity, or familial testing). Respond only regarding DNA mixtures (not single-source samples).

Names of commercial manufacturers are included for systems that are frequently used in laboratories; inclusion does not imply endorsement by the study team.

- *Does your laboratory conduct DNA mixture analysis and report the results in English?*
  - *Yes*
  - *No (Not eligible for study)*
- *By participating in this study, you affirm that*

  1) You will use the same diligence in performing these analyses as used in casework.
  2) Your responses to all surveys are accurate.
  3) You will not share or redistribute the electropherograms obtained during this study.
  4) At the completion of the study, you will destroy all electropherograms obtained as a part of this study.
  - *I agree on behalf of my laboratory, and my laboratory will abide by all four of these conditions*
  - *I do not agree and understand that we cannot participate (Not eligible for study)*
- *Please provide your laboratory name*
- *What type of laboratory do you represent?*
  - *U.S. Local laboratory*
  - *U.S. State laboratory*
  - *U.S. Federal laboratory*
  - *U.S. Private laboratory*
  - *Non-U.S. Local laboratory*
  - *Non-U.S. State/Provincial laboratory*
  - *Non-U.S. Federal/National laboratory*
  - *Non-U.S. Private laboratory*
- *How many DNA analysts conduct mixture analysis in your laboratory? (For the purposes of this study, a DNA analyst is an individual who has completed training and is qualified by your laboratory to conduct independent operational mixture casework.)*
  - *1*
  - *2-10*
  - *11-25*
  - *26-50*
  - *51+*
- *Please indicate all autosomal STR amplification kit(s) that are validated and used in your laboratory for DNA mixtures (check all that apply). For each, please specify all validated amplification cycle settings specified by your SOPs (separate multiple values with commas).*

- *Applied Biosystems AmpFLSTR Identifiler (specify amp cycles)*
- *Applied Biosystems AmpFLSTR Identifiler Plus (specify amp cycles)*
- *Applied Biosystems AmpFLSTR Profiler (specify amp cycles)*
- *Applied Biosystems AmpFLSTR Profiler Plus (specify amp cycles)*
- *Applied Biosystems GlobalFiler (specify amp cycles)*
- *Promega Powerplex 16 (specify amp cycles)*
- *Promega Powerplex 16 HS (specify amp cycles)*
- *Promega Powerplex Fusion 5C (specify amp cycles)*
- *Promega Powerplex Fusion 6C (specify amp cycles)*
- *Qiagen Investigator 24plex (specify amp cycles)*
- *Other (specify name and version)*

- *Please indicate all CE instrument(s) are validated and used for mixture casework in your laboratory (check all that apply). For each, please specify all validated voltage and injection time settings specified by your SOPs (separate multiple values with commas).*
  - *Applied Biosystems 3100 (specify voltage(s) and injection time(s))*
  - *Applied Biosystems 3130 (specify voltage(s) and injection time(s))*
  - *Applied Biosystems 3130xl (specify voltage(s) and injection time(s))*
  - *Applied Biosystems 3500 (specify voltage(s) and injection time(s))*
  - *Applied Biosystems 3500xl (specify voltage(s) and injection time(s))*
  - *Applied Biosystems 3700 (specify voltage(s) and injection time(s))*
  - *Applied Biosystems 3730 (specify voltage(s) and injection time(s))*
  - *Promega Spectrum (specify voltage(s) and injection time(s))*
  - *Other (specify name)*