General Guidelines for DNA Casework

1 Laboratory organization

1.1 To minimize the potential for carry-over contamination, the laboratory is organized so that the areas for DNA extraction, PCR set-up, and handling amplified DNA are physically isolated from each other.

1.2 Based on need, microcentrifuge tube racks have been placed in sample handling areas. These racks should only leave their designated area to transport samples to the next designated area. Immediately after transporting samples, the racks should be cleaned and returned to their designated area.

1.3 Dedicated equipment such as pipettors should not leave their designated areas. Only the samples in designated racks should move between areas.

1.4 Analysts in each work area must wear appropriate personal protective equipment (PPE). Contamination preventive equipment (CPE) must be worn where available. All PPE and CPE shall be donned in the bio-vestibules.

1.5 Required PPE and CPE for each laboratory are posted conspicuously in each bio-vestibule.

ARCHIVED

2 Work Place Preparation

2.1 Apply 10% bleach followed by water and/or 70% Ethanol to the entire work surface, cap opener, pipettes, and compu addter keyboard/mouse (when appropriate).

2.2 Obtain clean racks and cap openers, and irradiated microcentrifuge tubes, from storage. **Arrange work place to minimize crossover.**

2.3 Position gloves nearby with 10% Bleach/70% Ethanol/water in order to facilitate frequent glove changes and cleaning of equipment.

3 Microcentrifuge tube and pipette handling

3.1 All extraction tubes must be irradiated prior to use.

3.2 Avoid splashes and aerosols. Centrifuge all liquid to the bottom of a closed microcentrifuge tube before opening it.

3.3 Avoid touching the inside surface of the tube caps with pipettors, gloves, or lab coat sleeves.

© NYC OFFICE OF CHIEF MEDICAL EXAMINER

3.4 Use the correct pipettor for the volume to be pipetted. For pipettors with a maximum volume of 20µL or over, the range begins at 10% of its maximum volume (i.e., a 100µL pipette can be used for volumes of 10-100µL). For pipettors with a maximum volume of 10µL or under, the range begins at 5% of its maximum volume (i.e., a 10µL pipette can be used for volumes of 0.5-10µL).

3.5 Filter pipette tips must be used when pipetting DNA and they should be used, whenever possible, for other reagents. Use the appropriate size filter tips for the different pipettors; the tip of the pipette should never touch the filter.

3.6 Always change pipette tips between handling each sample.

3.7 Never “blow out” the last bit of sample from a pipette. Blowing out increases the potential for aerosols, this may contaminate a sample with DNA from other samples. The accuracy of liquid volume delivered is not critical enough to justify blowing out.

3.8 Discard pipette tips if they accidentally touch the bench paper or any other surface.

3.9 Wipe the outside of the pipette with 10% bleach solution followed by a 70% ethanol solution if the barrel goes inside a tube.

4 Sample handling

4.1 Samples that have not yet been amplified should never come in contact with equipment in the amplified DNA work area. Samples that have been amplified should never come in contact with equipment in the unamplified work area.

4.2 The DNA extraction and PCR setup of evidence samples should be performed at a separate time from the DNA extraction and PCR setup of exemplars. This precaution helps to prevent potential cross-contamination between evidence samples and exemplars.

4.3 Use disposable bench paper to prevent the accumulation of human DNA on permanent work surfaces. 10% bleach followed by 70% ethanol should always be used to decontaminate all work surfaces before and after each procedure.

4.4 Limit the quantity of samples handled in a single run to a manageable number. This precaution will reduce the risk of sample mix-up and the potential for sample-to-sample contamination.

4.5 Change gloves frequently to avoid sample-to-sample contamination. Change them whenever they might have been contaminated with DNA and whenever exiting a sample handling area.

4.6 Make sure the necessary documentation is completely filled out, and that the analyst’s ID is properly associated with the notations.

5 Body fluid identification

ARCHIVED

© NYC OFFICE OF CHIEF MEDICAL EXAMINER

5.1 The general laboratory policy is to identify the stain type (i.e., blood, semen, or saliva) before individualization is attempted on serious cases such as sexual assaults, homicides, robberies, and assaults. However, circumstances may exist when this will not be possible. For example, on most property crime cases when a swab of an item is submitted for testing, the analyst will cut the swab directly for individualization rather than testing the swab for body fluid identification.

5.2 A positive screening test for blood followed by the detection of DNA in a real-time PCR assay is indicative of the presence of human blood.

5.3 High Copy Number (HCN) testing is performed when the samples have a quantitation value ≥10.0 pg/uL for YFiler (at least 100 pg per amp), ≥20 pg/µL for Identifiler 28 cycles (at least 100 pg per amp), ≥ 5 pg/µL for Fusion (at least 37.5 pg per amp) or ≥10 pg/uL for Minifiler (at least 100pg per amp).

6 DNA Extraction Guidelines

6.1 Slightly different extraction procedures may be required for each type of specimen. Due to the varied nature of evidence samples, the user may need to modify procedures.

6.2 All tube set-ups must be witnessed/ confirmed **prior** to starting the extraction.

6.3 Use lint free wipes or a tube opener to open tubes containing samples; only one tube should be uncapped at a time.

6.4 When pouring or pipetting Chelex solutions, the resin beads must be distributed evenly in solution. This can be achieved by shaking or vortexing the tubes containing the Chelex stock solution before aliquoting.

6.5 For pipetting Chelex, the pipette tip used must have a relatively large bore – 1 mL pipette tips are adequate.

6.6 Be aware of small particles of fabric, which may cling to the outside of tubes.

6.7 With the exception of the Mitochondrial DNA Team, two extraction negative controls (E-neg) must be included with each batch of extractions to demonstrate extraction integrity. The first E-Neg will typically be subjected to the same testing as the extraction samples. The second E-Neg will ensure that the samples in that extraction set can be sent on for further testing in another team or in a future kit if the first E-neg is consumed. In the Mitochondrial DNA Team, only one extraction negative control is needed.

6.7.1 The extraction negative control contains all solutions used in the extraction process but no biological fluid or sample. For samples that will be amplified ensure the extraction negative passes based on the current Quantitation protocol.

ARCHIVED

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.
© NYC OFFICE OF CHIEF MEDICAL EXAMINER

6.8 If a sample is found to contain less than 20 pg/µL of DNA, then the sample should not be amplified in Identifiler (28 cycles); if a sample is found to contain less than 5 pg/µL of DNA, then the sample should not be amplified in Fusion; if a sample is found to contain less than 10 pg/µL of DNA, then the sample should not be amplified in YFiler; if a sample is found to contain less than 10 pg/µL of DNA, then the sample should not be amplified in MiniFiler.

6.9 Samples that cannot be amplified may be re-extracted, reported as containing insufficient DNA, or in some instances concentrated using a Microcon (see The Microcon DNA Fast Flow Procedure of the STR manual). The interpreting analyst shall consult with a supervisor to determine how to proceed. Other DNA samples may also be concentrated and purified using a Microcon if the DNA is suspected of being degraded or shows inhibition or background fluorescence during quantitation.

6.10 After extraction, the tubes containing the unamplified DNA should be transferred to a box and stored in the appropriate refrigerator or freezer. The tubes should not be stored in the extraction racks.

6.11 All tubes must have a LIMS label and/or the complete case number, sample identifier and IA initials on the side of the tube. This includes aliquots submitted for quantitation.

7 Controls for PCR analysis

7.1 The following controls must be processed alongside the sample analysis:

7.1.1 A positive control is a DNA sample where the STR alleles for the relevant STR loci are known. The positive control tests the success and the specificity of the amplification, and during the detection and analysis stage the correct allele calling by the software.

7.1.2 An extraction negative control consists of all reagents used in the extraction process and is necessary to detect DNA contamination of these reagents. **Note:** Since the Y STR system only detects male DNA, one cannot infer from a clean Y STR extraction negative the absence of female DNA. Therefore, an extraction negative control originally typed in Y STRs must be retested if the samples are amped in Identifiler or Fusion.

7.1.3 Samples that were extracted together should all be amplified together, so that every sample is run parallel to its associated extraction negative control.

7.1.4 An amplification negative control consists of only amplification reagents without the addition of DNA, and is used to detect DNA contamination of the amplification reagents.

7.2 Failure of any of the controls does not automatically invalidate the test. Under certain circumstances it is acceptable to retest negative and positive controls. **See STR Results Interpretation Procedure for rules on retesting of control samples (Identifiler) or STR Results Interpretation – Powerplex^{R} Fusion & STRmix^{TM} for Fusion.**

ARCHIVED

© NYC OFFICE OF CHIEF MEDICAL EXAMINER

8 Concordant analyses and "duplicate rule"

8.1 The general laboratory goal is to evaluate typing results to identify sample mix-up or any typing results that are in conflict with the context of the case. If either of those situations are encountered, it may be necessary to perform additional testing. Most often, that additional testing will take the form of repeating the DNA testing for a particular sample with a separate extraction and/or aliquot, amplification, and electrophoresis plate.

8.2 The reporting analyst will evaluate any DNA typing results in a case and determine if the results make logical sense in the context of the case. In many instances, this is demonstrated by concordance of typing results. For example:

- a series of bloodstains from a homicide scene match the victim
- mixtures in a sexual assault case are consistent with the victim and the same male donor(s)
- a set of samples from a gun show similar alleles in the mixtures

8.2.1 Some evidence types may not be expected to have concordant typing results. For example:

- samples collected from a variety of locations at a burglary scene
- numerous beverage containers collected from a bar
- samples taken from a variety of discarded clothing items from a shooting scene

9 PROCEDURE

9.1 EVIDENCE SAMPLES

9.1.1 Evaluate typing results and any match, inclusion or exclusion within the case. Any additional testing takes the form of a separate aliquot, amplification and electrophoresis run. The following guidelines apply:

9.1.1.1 Identical single source DNA profiles among at least two items (two evidence samples or one evidence sample plus an exemplar) within a case are considered internally concordant results.

9.1.1.2 If a sample does not match any other sample in the case and does not make sense in the context of the case, it must be repeated with a second amplification. If the only result was obtained using Y-STRs, this must be repeated in the Y system.

9.2 MIXTURE SAMPLES

ARCHIVED

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.
© NYC OFFICE OF CHIEF MEDICAL EXAMINER

9.2.1 If the sample consists of a **mixture of DNA**, several scenarios must be considered. Further analysis steps have to be decided based on the nature of each case. Consult with your supervisor if you encounter a situation that is not represented in the following examples:

9.2.1.1 If the alleles in a mixture are consistent with coming from any of the known or unknown samples in the case, e.g. a victim and a semen source, no further testing is needed.

9.2.1.2 If two or more mixtures in a case are consistent with each other and display substantially the same allele combinations, they are considered concordant.

9.2.1.3 If mixtures are found within a case that are consistent with the context of the case, it is acceptable for them not to be concordant. For example:

9.2.1.3.1 Mixtures of DNA found on touched items in a home invasion.

9.2.1.4 Discarded items in any kind of case.

9.2.2 Inconclusive samples and minor components of mixed samples do not require additional testing.

9.3 EXEMPLAR SAMPLES

9.3.1 Evaluate typing results and any match, inclusion or exclusion within the case, or between a suspect and an evidence case. Any additional testing takes the form of a separate extraction, aliquot, amplification and electrophoresis run. The additional testing must start with a second independent extraction, with the exemplar cut and submitted for extraction at a different time. If there is no additional exemplar material available for extraction, the additional testing may begin at the amplification stage.

9.4 ELIMINATION SAMPLES

9.4.1 Non-victim elimination exemplars (such as consensual partners, homeowners, business employees) do not require additional testing.

9.5 VICTIM EXEMPLAR

9.5.1 Additional testing of **victim's exemplar** is required if it does not match any of the DNA profiles of evidence samples in the case, including mixtures, and the case did not meet any of the criteria listed above.

9.5.2 Additional testing of a victim's exemplar is not required in the following situations:

ARCHIVED

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.
© NYC OFFICE OF CHIEF MEDICAL EXAMINER

9.5.2.1 A case which contains only samples that are insufficient for amplification and/or had no DNA alleles detected in evidence samples.

9.5.2.2 A case which contains only samples which are inconclusive/not suitable for comparison.

9.5.2.3 There is no reasonable expectation to detect the victim's DNA on an item of evidence, e.g. a crime where a hat was seen being dropped by fleeing suspect.

9.5.2.4 A case with a female victim where the samples processed yielded only male DNA profiles, or a case with a male victim where the samples yielded only female DNA profiles.

9.5.2.5 If the DNA profile of a **victim's exemplar** matches any of the DNA profiles of evidence in the case, or is present in a mixture.

9.6 SUSPECT EXEMPLAR

9.6.1 Additional testing of a suspect's exemplar (true exemplar **or** pseudo exemplar) is required if testing results in a "cold hit" to an evidence sample in the local database. A re-cut of the evidence should be initiated to confirm the match, or a repeat amplification if the exemplar sample has been consumed. This may require requesting evidence back to the lab for testing if it has previously been returned to the Evidence Unit. In such instances, email the Evidence Unit as well as DNA Sign-In detailing the nature of the request so that the evidence return can be expedited.

9.6.2 Additional testing of a suspect's exemplar is not required in the following situations:

9.6.2.1 If the DNA profile of a suspect's exemplar does not match any of the DNA profiles in the cross-referenced case or in the local database, 2) If a suspect exemplar is submitted to the laboratory for testing following a CODIS offender match and subsequent testing matches the offender profile.

9.6.2.2 If a suspect exemplar is submitted to the laboratory for testing following the testing of a pseudo exemplar and the profiles match.

9.6.2.3 If a suspect exemplar is submitted for comparison to a particular case and matches that case (or is included in a mixture seen in that case).

9.6.3 Since additional testing of exemplars is performed to confirm the overall conclusion (match, exclusion, or inclusion in a mixture), a partial DNA profile (at least one complete locus) that demonstrates the overall conclusion is sufficient.

ARCHIVED

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.
© NYC OFFICE OF CHIEF MEDICAL EXAMINER

9.7 PSEUDO EXEMPLARS

9.7.1 Pseudo exemplars do not require additional testing, with the exception of those that result in a “cold hit” to an evidence sample (see additional testing procedure in SUSPECT EXEMPLAR section above). Detection of a mixture on a pseudo-exemplar makes the sample inconclusive; no further testing is required.

10 Y-STR TESTING

10.1 Additional Y-STR testing is required in the following situations:

10.1.1 If the only result was obtained using Y-STRs, this must be repeated in the Y system.

NOTE: additional testing, other than that described above, may be performed at the discretion of the reporting analyst, if there is concern that a sample mix-up occurred, that there may contamination from a source in or out of the laboratory, or typing results otherwise appear to be in conflict with the context of the case other than the examples above.

10.2 Additional Y-STR testing is not required in the following situations:

10.2.1 If the concordance policy has been met with Y-STR typing results.

10.2.2 If the Y-STR results are concordant with the autosomal results: agreeing with an exclusion or inclusion, confirming the presence of male DNA, and/or confirming the number of male donors.

11 Sample contamination policy

11.1 Contamination is defined as the addition of exogenous DNA/biological fluid to evidence items, samples, or controls subsequent to the incident. Sources of contamination could be first responders, EMT’s, crime scene technicians, MLI’s, ME’s, ADA’s, NYPD personnel, or laboratory personnel.

NOTE: If the circumstances of the incident are such that the presence of DNA from someone other than a victim, witness, or perpetrator may be reasonably expected, that is not considered contamination. An example is the DNA of a police officer on a weapon that she/he removed from a suspect with bare hands during arrest. An exemplar received from such a person is considered as a routine elimination sample, not a sample submitted specifically to investigate potential contamination.

11.2 Contamination is identified in a variety of ways:

11.2.1 When a DNA profile from an evidence sample is compared to the laboratory’s LabTypes database in LDIS and a match is found to an individual represented there.

ARCHIVED

© NYC OFFICE OF CHIEF MEDICAL EXAMINER

11.2.2 When a DNA profile from an evidence sample is compared to the forensic databases in LDIS and a match is found there and subsequent examination of the case records reveal that it is possible that the evidence was collected by the same NYPD personnel, processed by the same NYPD laboratory criminalist, or the samples were processed together during DNA testing.

11.2.3 When a DNA profile, or alleles, are seen in a control sample. If a negative or positive control contains exogenous DNA, all the associated samples are deemed inconclusive.

11.2.3.1 Follow the procedures in the Forensic Biology Protocols for Forensic STR Analysis to evaluate potential contamination in negative or positive controls.

11.2.3.2 If contamination in negative or positive controls is confirmed, the assay fails and the associated samples are deemed inconclusive.

11.2.3.3 Any associated samples should be re-extracted or re-amplified, if possible.

11.2.3.4 If re-extraction or re-amplification of the sample is not possible, then use the appropriate statements in the template report to report the results of the associated samples.

11.2.3.5 The Control of Non-Conforming Work procedure in the Quality Assurance/ Quality Control Manual should be followed to document the incident.

ARCHIVED

11.3 The source of any exogenous DNA should be identified so that samples can be properly interpreted. It may be possible to identify the source by:

11.3.1 Examining other samples from the same batch for similar occurrences.

11.3.2 Examining samples from different batches, handled or processed at approximately the same time for possible similar occurrences (such as from dirty equipment or surfaces).

11.3.3 Processing samples submitted specifically to investigate potential contamination in specific case(s), e.g. from NYPD personnel.

11.4 If contamination within a sample has been discovered, the first step is to try to find an alternate sample.

11.4.1 As appropriate, a new extraction, amplification, or electrophoresis of the same sample can serve as an alternate for the affected sample.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.

© NYC OFFICE OF CHIEF MEDICAL EXAMINER

11.4.1.1 All case notes related to the discovery of exogenous DNA are retained in the case record. A Not Suitable for Comparison/Inconclusive Form is added to the case record that identifies the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

11.4.1.2 For this type of alternate sample the discovery of exogenous DNA is not noted in the report.

11.4.1.3 The Control of Non-Conforming Work procedure in the Quality Assurance/ Quality Control Manual should be followed to document the incident.

11.4.2 If there are other samples from the incident which would serve the same purpose, they could be used as an alternate sample. For example, in a blood trail or a blood spatter, another sample from the same trail/spatter should be used. Another swab or underwear cutting should be used for a sexual assault.

11.4.2.1 All case notes related to the event are retained in the case record. A Not Suitable for Comparison/Inconclusive Form is created that identifies the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

11.4.2.2 For this type of alternate sample, the discovery of the exogenous DNA is noted in the report for the contaminated sample(s). Use the appropriate statements in the template report to report the results of the contaminated sample(s). No names for the possible source(s) of the exogenous DNA are listed in the report.

11.4.2.3 The Control of Non-Conforming Work procedure in the Quality Assurance/ Quality Control Manual should be followed.

11.5 If an alternate sample cannot be found, then as a last resort, the samples containing the contaminant DNA can be interpreted, but must be done so carefully. The contaminant DNA profile may be used as a conditioned sample in a STRmix™ analysis to aid in the mixture deconvolution in accordance to the following procedure:

11.5.1 A STRmix™ LR calculation for the contaminant DNA profile must be performed before conditioning.

11.5.2 If the resulting LR > 1,000 (above the uninformative range), the contaminant DNA profile can be used as a conditioned profile in a subsequent STRmix™ deconvolution.

11.5.2.1 The original deconvolution should be noted on a STRmix™ "Not Interpreted/Reported" form in the case file. The results and conclusions

ARCHIVED

© NYC OFFICE OF CHIEF MEDICAL EXAMINER

must be reported using the template report wording and indicate that the contaminant DNA profile was used as a conditioned profile to obtain results.

11.5.3 If the resulting STRmix™ LR ≤ 1,000, this STRmix™ analysis should be noted on a "Not Interpreted/Reported" form in the case file. The sample must not be conditioned upon and the samples cannot be interpreted any further.

12 Technical Deviations

12.1 Technical Deviations must be requested when standard courses of actions will not be followed (aka, a "planned deviation") or when standard operating procedures were not followed and the resulting data will be used in casework (aka, an "unplanned deviation"). The impact of the deviation must be thoroughly evaluated.

Examples:

- Incorrect elution volume selected for M48 run but did not affect the DNA extracted from the sample. Analyst would like to send the sample for further testing.
- Incorrect RPM's on a centrifuge set and used for a microcon assay but the eluant still filtered through the microcon. The Analyst would like to send the sample for further testing.
- Incorrect length of time for an incubation step in an extraction assay but it was determined that the assay still extracted DNA from the substrate. Analyst would like to send the sample for further testing.

12.2 Technical deviations should be a rarity, and are not intended to be a general occurrence. Where possible, the analysis should be re-done. If it has been determined that a deviation is necessary, the proposed deviation should be discussed with a supervisor and/or manager first to determine if re-testing or submitting a deviation request is the best course. Deviation requests are submitted to the appropriate technical leader for approval. Such requests must be accompanied by a sound scientific justification as to why, even though the technical procedure was not followed, it is acceptable to use the resulting data.

12.3 If a technical procedure was not followed, or an instrument or assay had a failure, and you are not intending to use the data for interpretation or for a subsequent assay, then a technical deviation is not necessary. A note in the batch or other documentation within the case file is sufficient.

Examples:

- Z-crash error results in a failed M48 batch; samples are recut.
- Failed negative control from an amplification; samples are re-amplified.

12.4 The mechanism to submit a technical deviation is through the LIMS deviations tram stop. While this tram stop is also used for evidence discrepancy forms as well as STR electrophoresis batch failures, neither of which is considered a technical deviation.

ARCHIVED

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.
© NYC OFFICE OF CHIEF MEDICAL EXAMINER

12.5 Technical deviations can only be approved by a technical leader. If the relevant technical leader is out of the office, approval of a technical deviation will have to wait until their return or be assigned to the other technical leader of the lab.

12.6 Depending on the complexity of the technical deviation, it may be necessary for the analyst requesting the deviation to meet with their supervisor, their manager and the relevant technical leader in order to discuss the planned deviation. The potential end result of the deviation and its impact on the case and/or reported results must be considered. Several meetings may be necessary during the implementation of the deviation in order to assess the results of the deviation, before proceeding to the next stage of testing. The need to meet, and the number of meetings needed, is entirely based on the complexity of the deviation.

12.7 **Technical deviations** are different than **non-conforming work**. Refer to the Control of Non-Conforming Work section of the Quality Assurance/Quality Control Procedures Manual for details on when to submit a non-conforming work form.

13 DNA storage

13.1 Store evidence and unamplified DNA in a separate refrigerator or freezer from the amplified DNA.

13.2 During analysis, all evidence, unamplified DNA, and amplified DNA should be stored refrigerated or frozen. Freezing is generally better for long term storage.

13.3 Amplified DNA is discarded after the GeneMapper analysis is completed.

13.4 DNA extracts are retained refrigerated for a period of time, then frozen for long-term storage.

ARCHIVED

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.
© NYC OFFICE OF CHIEF MEDICAL EXAMINER