General Guidelines for DNA Casework

Laboratory organization

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.

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.

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

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.

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

Work Place Preparation

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

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

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

Microcentrifuge tube and pipette handling

1. Microcentrifuge tubes, Microcon collection tubes, Dolphin tubes, and M48 tubes must be irradiated prior to use.

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

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

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).

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.

6. Always change pipette tips between handling each sample.

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.

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

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

Sample handling

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.

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.

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.

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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.

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.

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

Body fluid identification

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.

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.

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) or ≥10 pg/uL for Minifiler (at least 100pg per amp).

   High Sensitivity DNA testing (Identifiler 31 cycles) can be performed if samples have a quantitation value of less than 7.5 pg/µL (or 20 pg/µL) and greater than 1 pg/µL.

DNA Extraction Guidelines

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.

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

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

3. 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.

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

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

6. 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 a micro-con and will be consumed to ensure that an E-neg associated with each extraction set will be extracted concurrently with the samples, and run using the same instrument model and under the same or more sensitive injection conditions as the 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. In the Mitochondrial DNA Team, only one extraction negative control is needed.

   Refer to the end of this section for flow charts.

   The extraction negative control contains all solutions used in the extraction process but no biological fluid or sample. For samples that will be amplified in Identifiler (28 or 31 cycles), YFiler or MiniFiler, the associated extraction negative should be re-quantified to confirm any quantitation value of 0.2 pg/µL or greater.

7. 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 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.

   Samples that cannot be amplified may be re-extracted, reported as containing insufficient DNA, concentrated using a Microcon (see Section 3 of the STR manual), or possibly submitted for High Sensitivity testing. The interpreting analyst shall consult with a supervisor to determine how to proceed. Other DNA samples may also be concentrated and purified using a Microconif the

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DNA is suspected of being degraded or shows inhibition or background fluorescence during quantitation. Samples that are 1 pg/µL to 20pg/µL may be submitted for High Sensitivity testing with a supervisor's permission.

8. 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.

9. 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.

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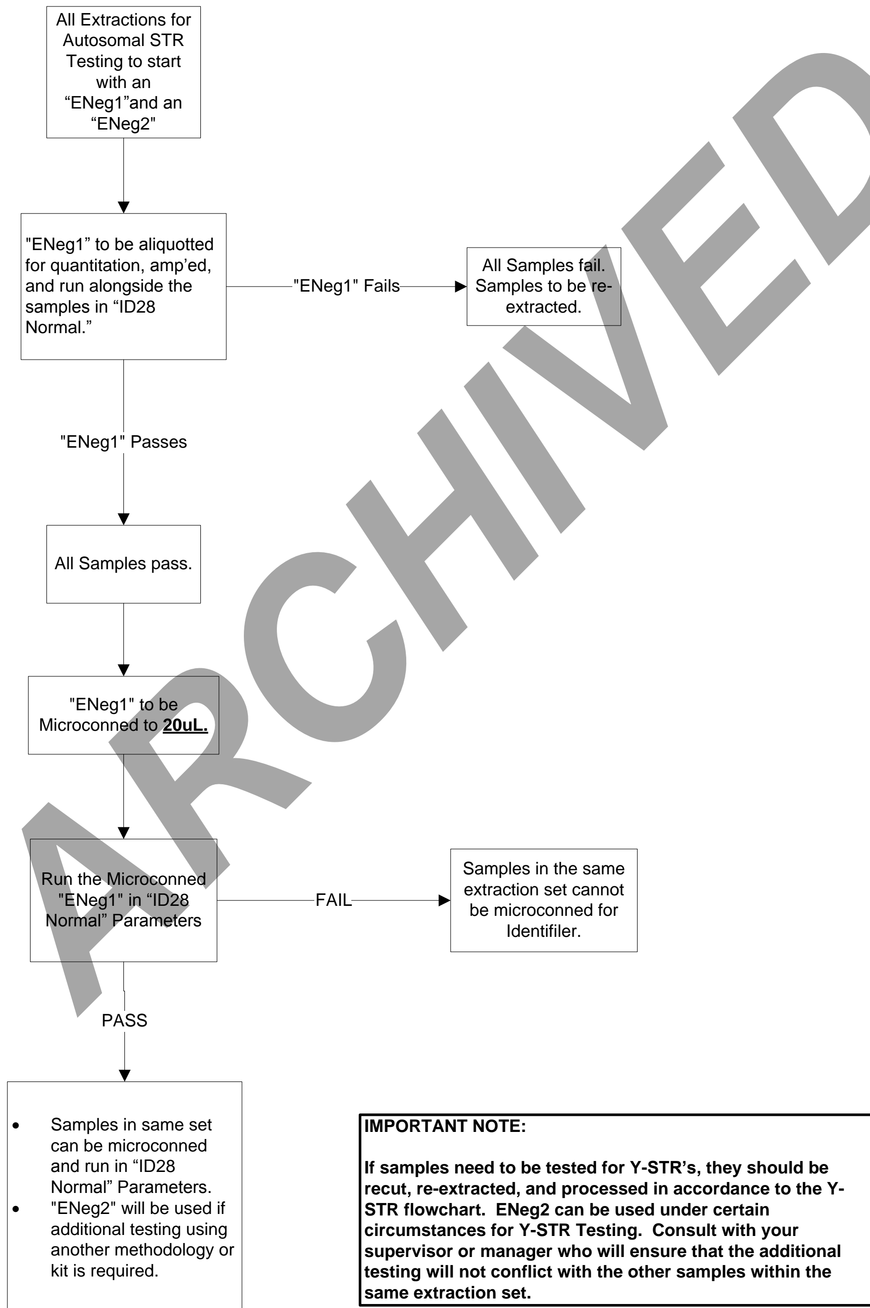
Extraction Negative Flow Charts

HSC and PC – EXTRACTION NEGATIVE FLOW AUTOSOMAL STR TESTING



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IMPORTANT NOTE:

If samples need to be tested for Y-STR's, they should be recut, re-extracted, and processed in accordance to the Y-STR flowchart. ENeg2 can be used under certain circumstances for Y-STR Testing. Consult with your supervisor or manager who will ensure that the additional testing will not conflict with the other samples within the same extraction set.

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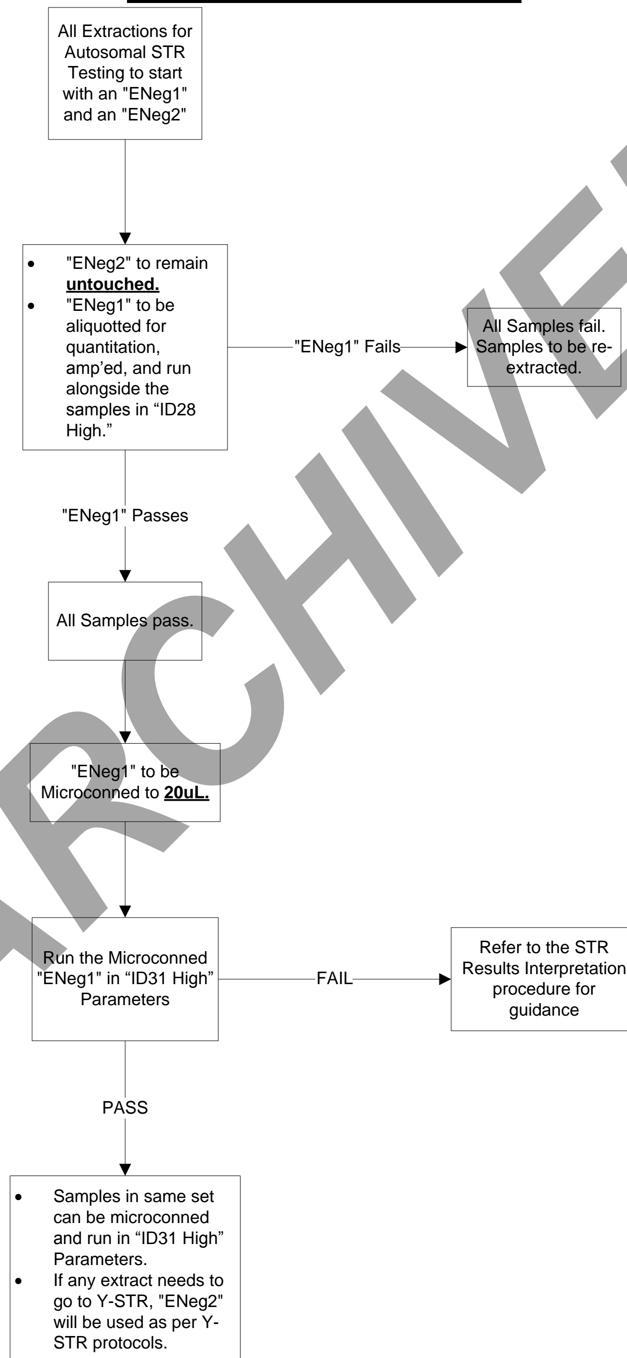
HYBRID – EXTRACTION NEGATIVE FLOW
AUTOSOMAL STR TESTING



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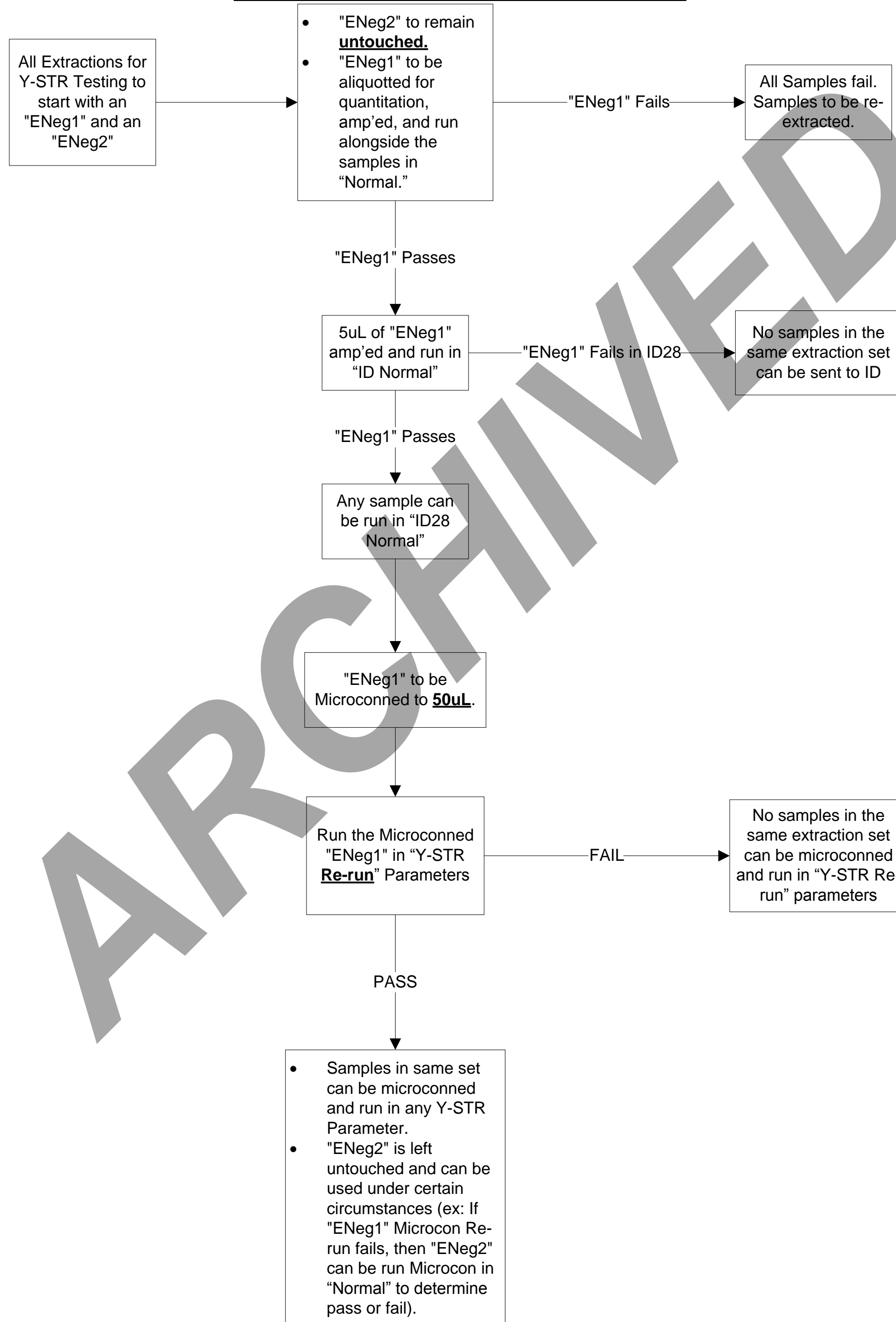
Y-STR TESTING (HSC, PC, and HYBRID) EXTRACTION NEGATIVE FLOW



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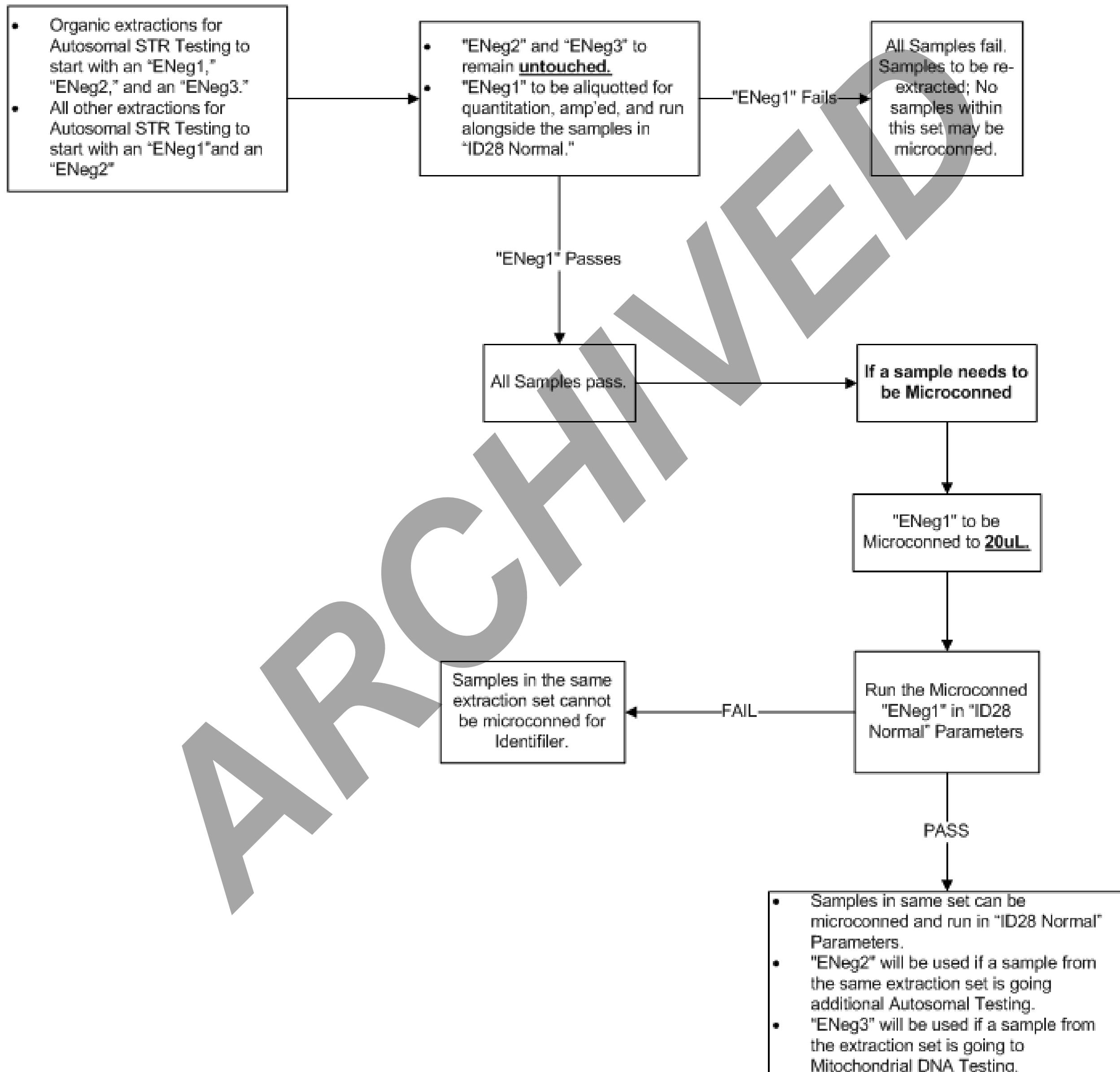
X-TEAM – EXTRACTION NEGATIVE FLOW
AUTOSOMAL STR TESTING



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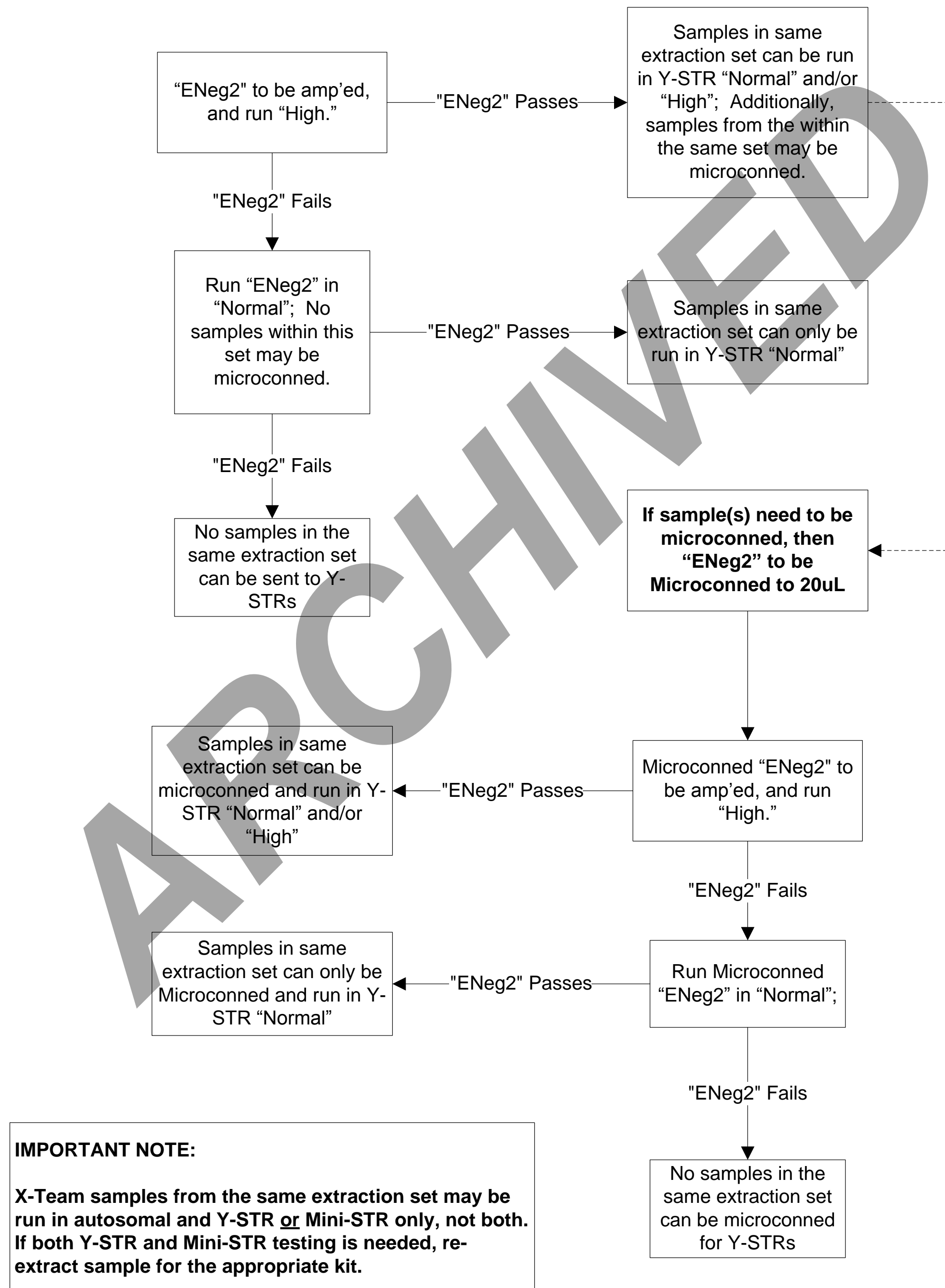
X-TEAM – EXTRACTION NEGATIVE FLOW
Y-STR TESTING



IMPORTANT NOTE:

X-Team samples from the same extraction set may be run in autosomal and Y-STR or Mini-STR only, not both. If both Y-STR and Mini-STR testing is needed, re-extract sample for the appropriate kit.

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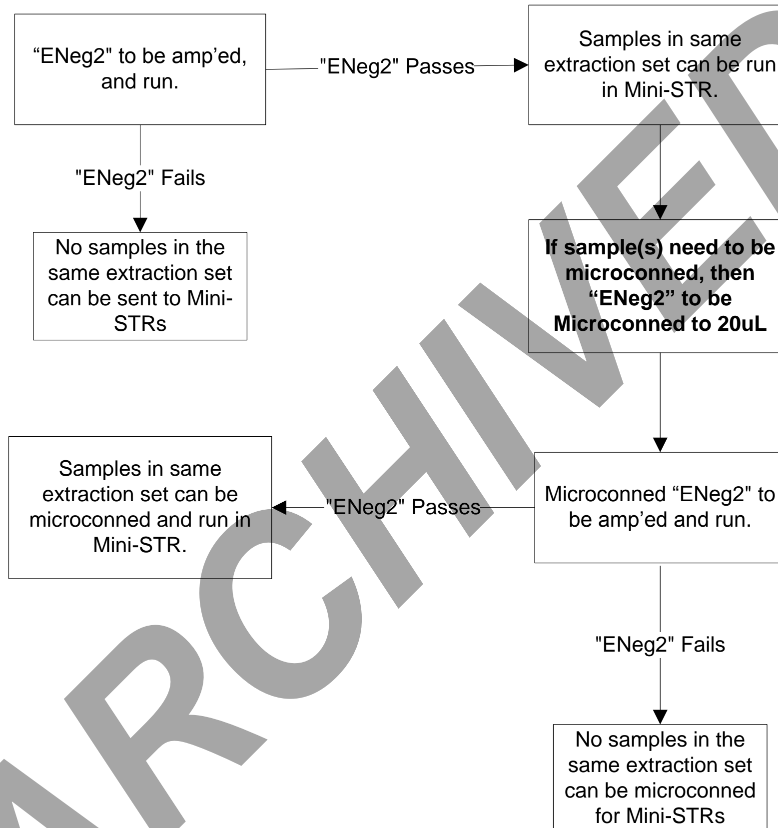
X-TEAM – EXTRACTION NEGATIVE FLOW MINI-STR TESTING



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IMPORTANT NOTE:

X-Team samples from the same extraction set may be run in autosomal and Y-STR or Mini-STR only, not both. If both Y-STR and Mini-STR testing is needed, re-extract sample for the appropriate kit.

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Controls for PCR analysis

The following controls must be processed alongside the sample analysis:

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.

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.

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

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.

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.**

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Concordant analyses and "duplicate rule"

The general laboratory policy is to confirm DNA results either by having concordant DNA results within a case, or by duplicating the DNA results for a particular sample with a separate extraction and/or aliquot, amplification, and electrophoresis plate. Concordant and duplicate analyses are used to detect sample mix-up (including false exclusions).

1. For evidence samples, concordance and/or duplication is designed to confirm a match or exclusion within a case or to detect sample mix up . The following guidelines apply:

   a. 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 ("duplicate rule").

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b. If a sample does not match any other sample in the case, it must be duplicated by a second amplification. If the only result was obtained using Y-STRs, this must be duplicated in the Y system.

c. 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:

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 concordance testing is needed.

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

3) If there is a sample in the case that results in a mixture of DNA and does not satisfy situation 1 or 2 above, the results need to be confirmed by a second amplification.

4) Consider duplicating mixed samples containing a low template amount of DNA (less than 250pg amplified).

5) Inconclusive samples and minor components of mixed samples that cannot be used for comparison (as defined in the STR Results Interpretation Procedure) do not require duplication.

d. Another method to satisfy this policy is if two different kits with overlapping loci are used. At least two (2) autosomal loci must be duplicated to confirm results. (For example, using Identifiler/MiniFiler on the same evidence sample.)

e. Automatic duplication designed to streamline testing of any evidence samples is also permitted.

2. For exemplar samples, duplication is designed to rule out false exclusions based on sample mix-up. Duplication must start with a second independent extraction, with the exemplar cut and submitted for extraction at a different time. The two resulting extracts must be aliquotted for amplification separately at different times, and aliquotted for electrophoresis separately and run on separate plates. If there is no additional exemplar material available for extraction, the duplication may begin at the amplification stage. For exemplars, the following guidelines apply:

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a. Duplication of a **victim's exemplar** is not required in the following situations:
   1) A negative case (no DNA alleles detected in evidence samples).
   2) A case which contains only samples which are inconclusive/not suitable for comparison.
   3) There is no reasonable expectation to detect the victim's DNA on an item of evidence, i.e. a crime where a hat was seen being dropped by fleeing suspect.
   4) A case with a female victim where the only samples processed yielded male DNA.
   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, the exemplar does not have to be duplicated.

b. If the DNA profile of a **victim's exemplar** 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 in a., the victim's exemplar must be duplicated to eliminate the possibility of an exemplar mix-up.

c. Since duplicate exemplar analyses are performed to confirm the exclusion, a partial DNA profile (at least one complete locus) that demonstrates an exclusion is sufficient.

d. Non-victim **elimination exemplars** (such as consensual partners, homeowners, business employees) will not be routinely duplicated. Duplication may be performed for specific cases, if necessary.

e. Duplication of a **suspect's exemplar** is not required in the following situations:

   1) If the DNA profile of a suspect's exemplar does not match any of the DNA profiles in the case, or in the local database, the exemplar does not have to be duplicated.

   2) If a suspect exemplar is submitted to the laboratory for testing following a CODIS offender match and subsequent testing matches the offender profile, the exemplar does not have to be duplicated.

   3) **Pseudo exemplars** do not have to be duplicated, regardless if the DNA profile matches any of the DNA profiles in the case. Detection of a mixture on a pseudo-exemplar should be confirmed with a rerun or reamp of the sample.

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4) If a suspect exemplar is submitted to the laboratory for testing following the testing of a pseudo exemplar and the profiles match, this serves as duplication.

f . If the DNA profile of a **suspect's exemplar** matches any of the DNA profiles in the case, or in the local database, and none of the criteria in e. are met, the suspect's exemplar must be duplicated to eliminate the possibility of an exemplar mix-up.

4. Partial profiles can satisfy the duplication policy. Consistent DNA typing results from at least one overlapping locus in a different amplification using the same kit is considered a concordant analysis.

5. For Y-STR testing, the sample does not have to be reamplified if the concordance policy/duplication rule has been met, or if the Y-STR results are concordant with the autosomal results: confirming an exclusion or inclusion, confirming the presence of male DNA, and/or confirming the number of male donors. Based on the case scenario it might be necessary to reamplify in order to confirm the exact Y-STR allele calls. There might not be sufficient autosomal data to establish concordance.

Exogenous DNA Policy

Exogenous DNA is defined as the addition of DNA/biological fluid to evidence or controls subsequent to the crime. Sources of exogenous DNA could be first responders, EMT's, crime scene technicians, MLI's, ME's, ADA's, NYPD personnel, or laboratory personnel.

1. Medical treatment and decontamination of hazardous materials are the first priority. Steps should be taken to minimize exogenous DNA as much as possible.

2. 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:

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

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

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c. Processing elimination samples to look for exogenous DNA occurring in the field or by laboratory personnel

Samples should be routinely compared to case specific elimination samples, personnel databases, and the local CODIS database for possible matches. Mixtures may have to be manually compared.

If a negative or positive control contains exogenous DNA, all the associated samples are deemed inconclusive and their alleles are not listed in the report. The samples should be re-extracted or re-amplified, if possible.

3. If a clean result cannot be obtained or the sample cannot be repeated then the summary section of the reports should state **"The following sample(s) can not be used for comparison due to quality control reasons."**

4. Once exogenous DNA has been discovered, the first step is to try to find an alternate sample.

   a. As appropriate, a new extraction, amplification, or electrophoresis of the same sample can serve as an alternate for the affected sample. For this type of alternate sample the discovery of exogenous DNA is not noted in the report. However all case notes related to the discovery of exogenous DNA are retained in the case file for review by the quality assurance group, forensic biology staff, attorneys and outside experts. A form is created that identifies the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

   b. If there are other samples from the crime scene 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 source should be used. Another swab or underwear cutting should be used for a sexual assault. In this scenario, the sample containing the exogenous DNA should be listed in the summary section of the report as follows: **"The [sample] can not be used for comparison because it appears to contain DNA consistent with a {NYPD member, OCME [laboratory] member, medical responder}. Instead please see [alternate sample] for comparison".** No names for the possible source(s) of the exogenous DNA are listed in the report. All case notes related to the event are retained in the case file for review by attorneys and their experts. A form is

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created that identifies the source of the exogenous DNA by Lab Type ID Number, if known, and stating which samples were affected.

5. If an alternate sample cannot be found then only samples containing a partial profile of the exogenous DNA can be interpreted. Interpreting samples containing a full profile of the exogenous DNA could lead to erroneous conclusions due to the masking effect of significant amounts of DNA.

    a. If a sample has a single source of DNA and this DNA appears to be exogenous DNA then the following should be listed in the summary section of the report: **"The [sample] will not be used for comparison because it appears to contain DNA consistent with a {NYPD member, OCME [laboratory] member, medical responder}."** No names for the possible source(s) of exogenous DNA are listed in the report. All case notes related to the event are retained in the case file for review by the quality assurance group, forensic biology staff, attorneys, and outside experts. A form is created that identifies the source of the exogenous DNA by Lab Type ID Number and stating which samples were contaminated.

    b. If a sample contains a mixture of DNA and **ALL** of the alleles from the source of the exogenous DNA appear in the mixture then the following should be listed in the summary section of the report. **"The [sample] contains a mixture of DNA. The mixture is consistent with a {NYPD member, OCME [laboratory] member, medical responder} and at least [#] other individual(s)." The [sample] will not be used for comparison."** No names for the possible source(s) of exogenous DNA are listed in the report. All case notes related to the event are retained in the case file for review by the quality assurance group, forensic biology staff, attorneys, and outside experts. A form is created that identifies the source of the exogenous DNA by Lab Type ID Number and stating which samples were affected.

Technical Deviations

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:

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- 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.

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.

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.

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.

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.

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

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need to meet, and the number of meetings needed, is entirely based on the complexity of the deviation.
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.

DNA storage

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

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

3. Amplified DNA is discarded after the Genotyper analysis is completed.

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

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