DNA Methods Manual

Administrative Guidelines

1 DNA Case Acceptance

1.1 Best Evidence

The DNA laboratory is committed to provide all law enforcement agencies in Ohio the best science on the best evidence in a timely fashion. The laboratory will follow the BCI DNA Evidence Submission Policy (LF-DNA-Submission Policy). The laboratory does not possess the resources to perform every analysis requested. Therefore, law enforcement agencies and prosecutors who submit resource-taxing and duplicative requests will be engaged in a discussion with the aim of arriving at a mutually acceptable plan of analysis.

If a forensic scientist is not able to reach an agreement with the law enforcement representative as to which items will be examined, the problem should be referred to DNA management.

2 Case Approach

2.1 The Role of the Methods Manual

The methods manual is a compilation of procedures and policies put in place as guides to the Forensic Biology(FB)/DNA examiners. They are not intended as a rigid, inflexible protocol, but rather as a guide to analytical procedures. The range of variation encountered in forensic casework evidence and circumstances requires flexibility in case approach, evidence handling, testing and interpretation. Deviations from the methods outlined in these manuals are permitted and at times necessary. The analyst's training incorporates an understanding of the chemical basis for the methods and for the tolerances of the systems. The peer and administrative reviews of casework serve as collaborative controls on methods usage. Therefore, deviations from the procedures outlined in this methods manual do not, by themselves, invalidate or compromise the testing results. Changes and updates in the manuals do not imply that previously issued methods and policies are incorrect or invalid.

2.2 Case Information

Obtain case information in the form of a synopsis, police report or documented oral description of the incident before a case is examined. The relationship of each evidence item to the crime scene should be known prior to examination. When a synopsis is obtained via telephone, make a detailed record of the conversation. Minimal information, but sufficient to support CODIS eligibility, is required for very simple cases such as a B&E with two swabs.

2.3 Choice of Items Examined

The forensic scientist should be familiar with the case information prior to beginning the analysis. The choice of which items to examine first and which tests to use is based on where the key evidence is likely to be found. If insufficient evidence is found on the first items examined, additional potentially key items ~~may~~ *should* be examined.

2.4 Combining Evidence Items

Distinct stains are generally not combined at collection because they may contain DNA from different sources. Separate items, even though packaged together, may still warrant separate collection—for instance swabbing of multiple gloves packaged together. In some situations, it may be necessary to combine items due to low DNA yield and the appropriateness of doing so is judged on a case-by-case basis.

2.5 Contents of Case Notes

Handwriting must be easily readable. The level of detail should, in conjunction with the procedure manual, permit replication of the analysis. Avoid excess and repetitive verbiage. Case notes must document all items that were received for DNA testing, consumed, and identify items not examined.

Use photographs and/or drawings to indicate the size and location of body fluid stains whenever possible. A scale or ruler should be used in photographs unless the item is a standardized item of known size such as PSA or HemDirect testing cartridges. Some items may not require a photograph based on the item type and analysis requested. Examples of this may include cartridges, cartridge cases, cigarette butts, hair standards, fingernail scrapings; or other items for which photo documentation provides no additional informational value.

Software, such as PowerPoint and Foxit PDF Editor Pro, may be used to assist in photographic documentation. Annotation of photographs or the LIMS.net notes page containing the photograph will include at minimum the case number, item number, date, and analyst initials. Areas taken for swabbings and cuttings must be indicated or described in the notes. If there is a positive result for a test, then the result must be documented on the photo (annotated with software or handwritten on the printed photo for the case file). The goal is to provide a reference to easily identify the positive stains and the location on items. For larger items of evidence, a diagram indicating tested areas and positive results is sufficient along with an overview picture of the item.

2.6 Deviations from Protocol

Deviations from written methods and conventions are at times necessary and are permitted as circumstances dictate. Document deviations, as appropriate.

2.7 Dissemination of Case Information

Verify the identity of a caller or *source of email* before discussing privileged information. Preliminary DNA testing results may be reported verbally only after a review of all data to verify conclusions are in compliance to laboratory guidelines has been completed. In time-sensitive situations, a mini-tech review can be conducted on the parts of the testing to be reported. The reviewer must document in the conversation log which results have been reviewed and that a verbal release may be made. This review is not required for the dissemination of forensic biology results. All communications with customers must be documented in the case conversion.

2.8 Evidence Preservation

Evidence items and reference standard samples are stored in a secure area until they are returned to the submitting agency. There is no time limit for return of items.

Extracted DNA is stored in a secure area and retained by BCI. The individual or location receiving or transferring DNA extracts is documented in case notes with the date, initials, and location of the evidence. After DNA extraction, the extractor places batch DNA extracts in a lab refrigerator or freezer with documentation (location, date, and initials) on the Sample Manifest form. The robot operator, as documented with the date and location on the Tecan Batch Checklist form, maintains custody of DNA extracts during robot runs. DNA extracts are stored long term in secure freezers with documentation (location, date, and initials) on the Tecan Batch Checklist form. Similar custody records with the location, date, and initials are maintained in case notes during manual post-extraction testing. Custody transfers of DNA extracts outside of the lab (e.g., Y-STR testing or vendor lab) are documented with the LF-DNA Extract Transport form or case conversation in LIMS. Receipt by a vendor lab is not required on the transport form. If the case conversation is used to record a transfer, the name of the person that pulled the extracts, sample names, date/time pulled, and where the extracts are being shipped must be recorded. A shipping tracking number should be recorded, if available.

Amplified DNA and DNA dilutions are considered work product and can be disposed of at any time after technical and administrative reviews of the case are completed.

2.8.1 Slides and extracts

1. Analyst-prepared slides that are positive are not sub-exhibited in LIMS.net and will be returned within the parent item or separately. Analyst notes will clearly indicate when positive slides are retained with parent items. *If the positive slides are returned separate from the parent item, the slides should be sub-itemed in LIMS.net*.
2. Negative slides submitted to BCI, such as in sexual assault kits, are packaged safely and returned with the item. Analyst prepared negative slides may be discarded.
3. Positive sperm search or PSA extracts where the total amount of sample is limited are combined with the sample for DNA testing or sub-exhibited, otherwise, the extracts are discarded.

2.9 Failed Tests - Documentation

The case notes should document and explain unsuccessful/rejected testing attempts with the identity and date of the individual taking the action. For example, document on the reported injection included in the case file: “Sample X did not inject on run 10-10-06. Reinjected on 10-13-06 Initials/Date”. If the electropherogram from the failed injection is included in the case file, document directly on the page. Document when data are not used due to failed controls. The electropherogram not reported for reprocessed DNA reference standards do not require documentation beyond “NR” since the data are not considered a failed test.

2.10 Firearms

Swabbing firearms and related components will routinely be performed by a scientist from the DNA section or authorized scientists for DNA sample collection in other sections. See the Lab Quality Assurance manual for sample collection instructions.

2.11 Initial Evidence Examination

If stains are not readily visible on an evidence item, use the alternate light source when trying to locate semen, saliva or urine stains. An infrared (IR) camera may be used to help locate blood stains on dark surfaces.

2.12 Blood Identification

Blood identification may be accomplished through the use of presumptive and/or confirmatory tests. Which test to use depends on the circumstances of the case and is left to the discretion of the forensic scientist. Any of the tests described in this manual may be used. Usually, testing will be limited to a presumptive blood test. Confirmatory testing for human blood (HemDirect) will be performed on a limited basis, as case circumstances dictate.

2.13 Harassment by Inmate

1. Submitting agencies will be encouraged to clearly indicate the specific area on the evidence to be tested
    a. This may be accomplished via agency case documentation or by circling the area on the item of evidence.
    b. Evidence may be returned unworked to the submitting agency if no information is provided to indicate area on evidence needing tested.
    c. Testing for body fluids may include feces, urine, saliva, semen, and/or blood.
2. Biological screening shall include all associated serological confirmatory tests when able
    a. Testing for blood will utilize TMB and HemDirect.
    b. Testing for semen will utilize AP, Microscopy for Sperm Search, and PSA if needed.
3. A positive sample requires the result be observed in a photograph.
    a. This does not apply to Sperm Search slides – positive slides are retained.
    b. Results that cannot be reproduced in the photo are reported as negative. No photo enhancements are to be performed for documentation.
4. Negative results do not need to be photographed.
5. Controls shall be run concurrently with the evidence and will be included in the photo documentation of positive results, *except AP and TMB*.
    a. If a sample will be consumed during the testing, the controls must be run both prior to the test and again alongside the test
6. DNA analysis shall not be performed on Harassment by Inmate evidence.

2.14 Sexual Assault Evidence

2.14.1 Direct-to-DNA Approach for Sexual Assault Evidence

The direct-to-DNA approach acknowledges the greater sensitivity of current DNA methods relative to body fluid identification methods. Limited screening is performed prior to collecting sexual assault evidence samples for DNA testing. Additional body fluid identification may be performed as needed following DNA testing. Refer to the chart below for caregiver/cohabitation cases. **Below are general guidelines.**

1. Review case information

2. Choose samples most likely to contain body fluids foreign to the victim *for first round of testing*. *Intimate samples are defined as orifice and skin swabs from areas usually covered by clothing*.
    a. One assailant, no consent contacts, fewer samples. Generally, vaginal and anal samples are sufficient *for first round of testing*.
    b. Multiple or undetermined assailants and/or consent contact, choose more samples *for first round of testing*.
    c. If penile penetration is suspected, generally ~~screen~~ *send* orifice swabs forward for a differential DNA extraction. In certain circumstances, it may be useful to screen orifice swabs for AP as a target kit. ~~for AP and select the strongest swabs for DNA. If no clearly AP positive samples or AP testing not performed, send those indicated by case information. In non-caregiver cases for individuals 5 y.o. or older, samples can be sent forward for testing without AP screening.~~
    d. If digital reported, send the orifice sample indicated by case information, intimate skin stain, external genital and underwear swabs forward for *differential* DNA testing. No AP testing is needed.
    e. Oral assault (fellatio) reported or unknown AND collected within 24 hours: No AP testing is needed, collect samples for differential DNA testing.
    f. ~~Send AP positive orifice swabs and underwear for differential extraction.~~ *Send AP positive underwear and other collected samples for differential extraction. Report these as "positive for acid phosphatase activity." Older evidence may produce weaker AP reactions. ALS staining may also be used to target sampling. Oral only assaults may be non- differential for underwear.*
    g. ~~Older evidence may produce weaker AP reactions.~~
3. Intimate swabs and underwear with red-brown staining without documentation that the victim was menstruating should be TMB tested and the results reported.
4. Fingernail samples are not routinely examined. The necessity for fingernail sample examination is determined by the case facts.
5. Use targeted approach as applicable. For example, case information = vaginal ejaculation, one contributor expected, vaginal swab = strong AP. Send vaginal swab to DNA and may stop the screening process.
6. Do not perform amylase screening on swabs.
7. Swab and cutting guidelines
    a. Internal Vaginal (≥ 12 years old) and oral swabs (oral assault reported AND collected within 24 hours): cut one swab equivalent of swab shells taking equal sample amount from each swab.
    b. Vaginal (<12 years old), penile, anal, and other skin swabs: cut up to a two-swab equivalent of swab shells taking equal sample amount from each swab.
    c. Strong AP vaginal and anal: cut ONE swab equivalent of swab shells taking equal sample amount from each swab.
    d. Underwear: ALS and AP/Amylase mapping as needed. Take a cutting and/or swabbing from positive area. Attempt to take cuttings from inner layer only. Consider regional swabbings from the front panel, crotch, and back panel as needed for weak AP/AP negative and for digital penetration. Swabbings from the exterior waistband may be collected if the synopsis indicates a suspect removed/handled the underwear.

e. *Mons pubis: test prior to pubic hair combing. If foreign DNA detected but not usable and nothing else probative, run PHC if available*. Pubic hair *comb:* If used by the SANE, swab the trace debris/comb and consider an intimate sample.
f. Gauze: cut from ALS positive areas. If ALS negative or auto-fluorescing, a random cutting may be selected.

8. Subject hand*/penile* swabbings, when collected in a timely fashion, have evidence potential. DNA testing may be performed on these samples and no body fluid testing is required. *The relationship of the subject and victim should be considered.*

9. If the first round of DNA testing does not yield a probative result, additional samples should be tested in most cases especially from intimate areas.

General Case Approach

1. *Generally blind swabs submitted for a collection from an item do not need to be reported as "No stains for analysis". Notes should document the condition of evidence. If an item is submitted as "from a stain" or similar and no staining observed on the collection swabs, then it should be reported as "No stains for analysis". If no stains are observed on oral swabs, then document visual appearance of swabs, but do not need to be in report.*

Direct-to-DNA Approach Guidelines for Sexual Assault Evidence	
Scenario	***Action***
Caregiver/Cohabitation Cases (including child under 5)	*Follow guidelines from table in 2.15*
Orifice samples – consumed/not consumed	*Cut and send for DNA - differential extraction*
Targeted approach as applicable. Example, case info = vaginal ejaculation, one contributor expected, vaginal swab = strong AP. Send vaginal swab to DNA and may stop the screening process	*AP, cut and send for DNA if strong- differential extraction* *Collect and test additional samples if probative result not obtained.*
No oral assault known and/or more than 24 hours	*Don't collect oral swabs*
Skin stain swabs from intimate area (for penile or digital), pubic hair combing, or sites of ejaculation - consumed	*Cut and send for DNA - differential extraction, except breast unless unconscious/possible ejaculation site*
Skin stain swabs from intimate area, pubic hair combing, or sites of ejaculation - not consumed	*Cut and send for DNA - differential if intimate area and penile assault or loss of consciousness*
When a kit contains MANY skin stain swabs, the scientist may AP and not run all of the skin stain swabs if negative. Use the synopsis to help guide testing of skin stain swabs.	*Cut and send for DNA - differential if intimate area and penile assault*

Swabs of bruises, only test them in stranger cases.	*Cut and send for DNA – non- differential*
Pediatric physical abuse kits	*Approach on a case-by-case basis*
Neck swabs should be run in kits where strangulation is indicated; effective April 2023 strangulation adds an additional felony charge (ORC 2903.18).	*Cut and send for DNA – non- differential*
Skin stain swabs from non-intimate area - consumed or not consumed – consider case synopsis	*Cut and send for DNA – consider differential /non-differential*
Clothing items, tampons, sanitary pads	*Screen for AP at a minimum (if examining) unless known oral/digital assault only and no consent*
Condoms	*Swab and send for DNA - differential extraction – separate sample from inside and outside*
Intimate skin swabs - Changed and showered	*Cut and send for DNA – consider differential /non-differential*
UW - Changed and showered 0-2 days	Screen for AP, collect samples from front and back (AP + or -), separately - differential extraction
2+ days later, changed and showered – AP UW	Send AP positive samples forward for differential extraction

2. *If probative Globalfiler results are only found in non-intimate area samples and more intimate areas that may be consistent with penetration meet the criteria for Y-STR analysis, consider Y-STR testing. If a male DNA standard has not been submitted, add a Y-STR footnote to the conclusion for the intimate sample. If a male DNA standard has been submitted, a discussion with the investigator or prosecutor for the case may be helpful to determine if the Y-STR testing is needed for the more intimate sample. Y-STR testing will typically be performed for cases involving juveniles on eligible samples.*

2.14.2 Traditional screening

Full biology screening can be done on a limited basis or in a case-by-case circumstance where such analysis is warranted. Discuss case approach with DNA management prior to proceeding with full screening.

1. Microscopic evaluation of the slide produced at the hospital is sufficient for the identification of spermatozoa. If more than one hospital slide for each orifice is submitted, it is only necessary to examine one of the slides. Alternatively, make a separate slide from the extract of a combined portion of each swab from the orifice.

   a. If positive and case has limited evidence and sample remaining, dry the remaining extract and retain with the swabs.
   b. To dry extract, centrifuge at max speed for 3 minutes to pellet cell debris. Carefully pipette off most of supernatant and absorb pellet to swab. Dry swab, *subitem in LIMS*, and package with retained samples.
2. Perform AP testing on all orifice swabs if not previously tested. Examination of oral swabs may be omitted if:
   a. A clear and apparently reliable history indicates no oral assault (fellatio) has occurred; or
   b. 24 hours have elapsed between time of assault and time of collection.
3. Positive slide and positive/negative swab AP→ no further biological analysis
4. Negative slide and negative AP → negative for semen → no further biological analysis
5. Negative slide and positive AP → a PSA test may be performed based on case circumstances
6. QNC slide and positive/negative AP → a PSA test may be performed based on case circumstances
7. Refer to the report wording section.

2.15 Caregiver/Cohabitation Cases

Case information, such as time and activities since alleged assault, may impact testing choices. Consider further screening prior to DNA testing if a key sample will be consumed.

Situation	Suspect	FB Testing	Ys if male standard is submitted	Full Testing for Semen if Probative DNA Result will be Reported	Special Remark if tested with GF or Ys ?
Child less than 5 y.o. Intimate Swabs	Caregiver	AP or Full testing for semen (AP, p30, Sperm Search) prior to DNA if Consumed	Based on quant and GF data	Yes (AP, Sperm Search, p30) with double negative if GF or Ys probative	Yes, unless strong FB results. For example, sperm search rating at least +1 or strong PSA result.
Child less than 5 y.o. Intimate Swabs	Non-Caregiver	At least AP	Based on quant and GF data	No	No
Child 5 y.o. or older Intimate Swabs	Caregiver/Cohabitation	At least AP	Based on quant and GF data	No	Based on case information
Child 5 y.o. or older Intimate Swabs	Non-Caregiver	AP/Full semen testing optional	Based on quant and GF data	No	No
Clothing All/Diapers*	Caregiver/Cohabitation	AP or Full testing for semen (AP, p30, Sperm Search) prior to DNA if Consumed	Based on quant and GF data if FB positive. No Y testing for blind swabs.	Yes (AP, Sperm Search, p30) with double negative if GF or Ys probative	Yes
Clothing All/Diapers*	Non-Caregiver/Non-Cohabitation	AP/Full semen testing optional	Based on quant and GF data	No	No
Rape/Domestic Violence Non-Intimate Skin Swabs (e.g. neck)*	Non-Stranger/Caregiver/Cohabitation	AP/Full semen testing optional	Based on quant and GF data	No	Yes
Rape/Domestic Violence Non-Intimate Skin Swabs (e.g. neck)*	Stranger	AP/Full semen testing optional	Based on quant and GF data	No	No

*If no probative results from more intimate samples

The occurrence of incidental transfer of DNA between household members is well established. The circumstances surrounding the deposition of the male/caregiver/family member DNA herein cannot be determined based on DNA results.

2.16 Amylase Testing

The following are general guidelines. Additional testing may be performed based on case specific facts and analyst discretion.

-Amylase testing may be performed subsequent to obtaining foreign DNA results for suspect-on-victim oral assault and digital/penile penetration when no oral assault occurred or is unknown.
-Amylase testing may assist in stain identification on items such as ski masks.

2.17 Species Testing

No animal species testing is available at BCI. Human testing is generally not performed on samples that will go on to DNA analysis since the DNA tests are higher primate/human specific.

2.18 Cutting Guidelines Overview

These are guidelines. The range of variation encountered in forensic casework evidence and circumstances requires flexibility and analytical judgment.

Item	Findings	Amount to put into tube	Notes
SEMEN			
Orifice/skin swabs (see section 2.13)			
Condom	Any	1 swab from each side	Separate tubes
Underwear/pants/towels		1 x 1 cm and/or one swab	Collect swabbing from area around cutting. Test swabbing and cuttings separate.

AMYLASE			
Skin swabs (see section 2.13)			
Bra	No W/L test	1 swab	Swab inside cup (one swab), cover nipple area. Do each cup separately
Underwear	ALS typically may W/L map	1 x 1 cm from + areas. Swab entire area	Test swabbing and cuttings separate.
Bottle, Can, Straw	No W/L test	1 swab	Sample with one swab
Cig butt	No W/L test	1/4-1/2 filter paper. Avoid seam.	A swabbing of the cigarette butt may also be used for testing
Envelopes	No W/L test	1 swab or 1x1 cm from inner part of seal	Steam or soak open, swab flap and envelope glue area with one swab

Item	Findings	Amount to cut into tube	Notes
Touch/Wearer			
Knife handle, steering wheel, airbag	No testing	1-2 swab	Separate areas possibly handled/in contact with different individuals
Disposable gloves	No testing	1 swab	1 swab per side, e.g. 4 tubes per glove pair
Disposable glove tip	No testing	place tip in tube	Add SEB directly to tip
Gloves (with obvious int./ext.)	No testing	1 swab	1 swab per glove interior, e.g. interior right glove, interior left glove
Sweatshirt	No testing	4 swab shells in 4 tubes	Cuffs (separately), tag, inside back collar. Zipper pulls, draw strings, and cuttings from tags can also be tested.
Blood	Heavy red TMB+ stain	1/4 swab	
	Faint stain w TMB+	1 swab	
Blood on Clothing	Heavy red TMB+ stain	5 x 5 mm	Alternately swab, cut tip of swab into tube
	Weak TMB+ stain	1 x 1 cm	
	TMB+ Stain on denim/darkly dyed fabric	1 swab or 1x1 cm cutting	

Item	Findings	Amount to cut into tube	Notes
Miscellaneous			
Fingernail scrapings	No testing	swab entire stick or preferably test swabs in kit, ~~retain one swab equivalent~~	
Fingernail clippings	No testing	swab clippings or place clippings into tube	Add extraction buffer to clippings, agitate, remove clippings and retain.
Urine, bones, teeth, fetus, feces		Straight to DNA for sampling	
Hair	Root present	3-5 from SAME source - cut roots + 1 CM shaft.	SAVE REMAINING SHAFT

NOTES

1. One swab means the outer shell of the swab only with minimal unstained cotton.
2. On underwear, avoid heavily stained areas that may contain an abundance of wearer type. Consideration can be made if the area is also AP positive. These areas may be sampled for Y-STR testing.
3. If sampling one item into multiple tubes, label tubes distinctly and record in notes which samples are in which tubes.

3 Case Information Assembly

All standard forms and templates placed in the file must be of print shop quality—no crooked, ~~or~~ worn, or n^{th} generation copies. Current controlled examination documentation forms must be utilized in case files.

Handwritten notes must have a professional appearance, be easily readable and devoid of excessive cross-outs.

To facilitate efficient technical and administrative review, case file documents should be in the following order with #1 being on the top.

With LIMS.net and paperless case file records, generally electronic copies of forms will be used or stored in LIMS.net. Acceptable alternatives to the list below will be communicated to staff in emails until the next manual revision.

3.1 Uploaded to the case in LIMS.net (not part of the Notes Packet)

1. SANE notes and synopsis
2. Cross reference documents (report and electropherograms)
3. CODIS entry

3.2 LIMS.net Notes Packet

1. *Comments sheet, if applicable*
2. Standard ACS
3. Forensic sample notes and reagent log (if applicable)
4. Extraction manifest, Quantitation/Amplification set up record (Extraction batch 1, batch 2, etc.), and Evidence electropherograms as specified below:
    a. Manifest #1
    b. Workbook #1
    c. Manifest #2
    d. Workbook #2
    e. Differential #1
    f. Differential Blank #1
    g. Non-differential #1
    h. Non-differential Blank #1
    i. Differential #2
    j. Differential Blank #2
    k. Non-differential #2
    l. Non-differential Blank #2
5. Reference standard notes, if not documented in FB notes
6. Reference Standard electropherograms in numerical order
7. Popstats output/ArmedXpert output

8. Y-STR testing documentation/Y-STR statistical printouts

3.3 Electronic Data Organization

1. Standards Batch Folder
   - Tecan Batch Checklist .xls
   - Checklist/Quant/Batch/Controls .pdf
   - Quant .eds
   - Batch Workbook .xls
   - Run folders with .HID files
   - ACS.xls

2. Evidence Batch Folder
   - Extraction manifests.xls
   - Genemapper projects.ser
   - Tecan Batch Checklist .xls
   - Checklist/Quant/Batch/Log/Controls .pdf
   - Quant .eds
   - Batch Workbook .xls
   - Run folders with .HID files
   - Extraction Reagent Log .xls

If additional samples are analyzed **before** a report is issued, the data for additional analysis should be grouped together as stated above.

If analysis of additional samples is required **after** an initial report has been issued, the new documentation should be arranged in the same order as above in the Notes Packet for the new assignment.

Changes made to pdf batch files must be tracked. To address this, the pdf batch file will be saved with a version number starting with v1. Changes made to the original pdf document will be noted on the first page of the document and saved as the next version number, e.g., v2. Only the final version of the pdf document has to be signed by all reporting analysts.

3. In instances where a previously reported standard is amplified with the Globalfiler or Yfiler Plus kits for additional comparisons, the file paperwork will be minimal. The paperwork will be maintained in LIMS.net as described above with the assignment.

4 Abbreviations

The following abbreviations as well as those listed in the Lab QA manual may be used in case file notes:

~	about
✓	checked
(-)	negative
(+)	positive
( )	signal less intense
∴	therefore
?	unknown
als	alternate light source
Amel.	amelogenin
amp	amplification
amy	amylase
ap	acid phosphatase
as	anal swab
AT	analytical threshold
~~attn~~	~~attention~~
AX	ArmedXpert
bet	blue evidence tape
bld	blood
bp	back panel
bpb	brown paper bag
brn	brown
CC	cartridge case
creat	creatinine
ct	clear tape
cts	clear tape sealed
~~cont~~	~~containing~~
ded	deduced
det	detective
dh_2O	deionized/filtered water
DI	Degradation Index
diff	differential
dil	dilute
dk	dark
DNSFC	data not suitable for comparison
~~dsm~~	~~double slide mailer~~
EC	expected contributor
env	envelope
er	evidence receiving
et	evidence tape
exp	expires
ext	exterior
F1	non sperm fraction
F2	sperm fraction
fmRMP	forced modified RMP
~~FOL~~	~~Fruit of Loom~~
Fp	front panel
GF	Globalfiler
GUI	graphical user interface
Het	heterozygous
Hom	homozygous
hosp	hospital
ID	Identifiler
inj	injection
int	interior
IQ	DNA IQ extraction
kn	kit notes
l	left
lab	labeled
lg	large
lt	light
man	manila
mb	manipulation blank
me	manila envelope
med	medium
micro	microscopic exam
mj	major
mn	minor
mRMP	modified RMP
mv	minus victim
NDP	*no DNA profile*
nc	not collected
nd	not detected
ne	not examined
neg	negative
norm	normalization
np	not performed
nr	not reported
nt	not tested
nv	non-victim
obs	observed
odh	Ohio dept of health
oes	orange evidence sticker
ofc	officer
(O)NSE	(organic) non semen extraction
orig	original
os	oral swab
(O)SE	(organic) semen extraction (differential)
pb	paper bag
pg	page
PHC	pubic hair combing
PHR	peak height ratio
pkg	package
plb	plastic bag
pos	positive
pt	patient
prep	prepared
prev	previously
psa	prostate specific antigen
PU	pull-up
QNC	questionable, not called
r	right
RBS	*red brown stain*
rec'd	received
rRMP	restricted RMP
ret	red evidence tape
rets	red evidence tape sealed
RMP	random match probability
RP	re-processing
s	suspect
SEB	stain extraction buffer
sec	second
sm	small
sme	small manila envelope
ss	single source
~~ssm~~	~~single slide mailer~~
ST	stochastic threshold
std	standard
STR	short tandem repeat
sub	submitted
sw	swab
s/w	sealed with
T	tails
tmb	tetramethylbenzidine
TTP	tube to plate
uRMP	unrestricted RMP
Uro	urobilinogen
uw	underwear
v	victim
vag	vaginal
vol	volume
vs	vaginal swab
w/l	wurster/laux paper
we	white envelope
wk	weak
wpb	white paper bag
yet	yellow evidence tape
zlb	zip loc bag

5 Report Wording and Format

1. To the extent possible, reports should conform to the style and conventions produced by the report templates.
2. Misspellings, poor grammar, slang and potentially offensive words, even if used in the item descriptions at submission, should not be reproduced in the reports.
3. Prior to writing the report, verify that the submission sheet evidence descriptions are consistent with the format described in the lab quality assurance manual. The following examples provide clarity of importance as well as report writing compatibility. Problems with evidence descriptions should be referred to DNA management.
    a. Sexual assault kit from Jane Doe
    b. Bra from Jane Doe
    c. Swabbing of stain from window frame-point of entry
    d. Buccal swabs from John Smith
4. DNA cases must list all items for which body fluid/DNA examination was requested, test results/conclusions, and items not examined. Reports will include if samples are collected from the submitted items for possible testing. DNA reports with a previously issued forensic biology report must list all evidence items on which DNA testing was performed. The specific sub-items and cuttings versus swabbings should be listed in reports.
5. Report all chemical or DNA testing performed on evidence items, including both positive and negative results and DNA inclusions. Refer to the DNA section for guidelines regarding reporting of individuals excluded from samples.
6. ~~6.~~ Sexual assault kits may contain hospital slides prepared from swabs collected from body orifices. The slide and swabs from each orifice are submitted for possible testing. The case notes *must* ~~should~~ reflect all testing performed on the contents of each orifice sample ~~set~~. The report *must* ~~should~~ communicate the overall testing conclusion for the contents of each orifice sample set.
7. A table of results is not required to report allele calls.
8. Adhere to the report wording guidelines specified for each testing method which are populated in the report matrix in LIMS.net. Conclusions for each item should be entered into the report Matrix and reports generated from there. In rare instances, it may be appropriate to use additional verbiage for clarity.
9. Forensic Biology results once initially reported in a laboratory report (positive and negative) do not need to be re-reported in subsequent reports. Results from positive and negative forensic biology reports may be included in comprehensive reports.
10. Issue an Amended Copy of a report if a substantial error is discovered in a report after it has been issued (See Laboratory Quality Assurance Manual).
11. A cross referenced case may be listed under the case number as **Cross Reference 00-00000**.

Forensic Biology Methods

6 Use of the Alternate Light Source and Infrared Camera for Detection of Stains

6.1 Introduction

Dried stains such as saliva, semen, urine and perspiration often produce a bright fluorescence when exposed to ultraviolet light. This property can be utilized in the non-destructive examination of items for the presence of these body fluids through the use of an alternate light source that emits ultraviolet light.

The fluorescence in dried semen stains is thought to be due to non-proteinaceous compounds produced by a strain of the bacterium Pseudomonas fluorescens. Not every semen or saliva stain will fluoresce, so further testing of negative stains may be warranted. Also, other materials such as foods, cosmetics, etc., may fluoresce, so additional tests may be performed. Finally, depending on the color and/or fabric of the substrate, strong background fluorescence may be produced. In this case, experimentation with different wavelengths and colors of eye protection is warranted. BCI laboratories utilize multiple types of alternate light sources (ALS), the Spex Crimescope CS-16, the Crime-lites 80S and 80L, Crime-lite 82 Pro, Crime-lite ML, and Crime-lite ML2.

Blood does not emit fluorescence when exposed to ultraviolet light. Instead, blood will absorb infrared light. This property can be utilized in the non-destructive examination of evidence where it may be difficult to observe bloodstains with the naked eye. BCI laboratories utilize a reflected infrared photography system, where infrared light can be photographed as it is reflected off of the surface of an item. This will allow the observation of bloodstains on some dark or heavily patterned materials, as the blood will absorb the infrared light and remain dark, while the remainder of the item will reflect the infrared light back. Not all surfaces or materials will reflect infrared light and thus this is only considered another tool to be used in the detection of stains rather than a definitive test that will identify all potential stains. BCI laboratories utilize a Fujifilm HS-30 EXR Digital Camera, with the internal IR blocker removed and an attached IR filter [Peca 914 (89B)] and the Crime-lite ML2 for the use of IR photography.

6.2 Safety Considerations

High intensity xenon light source, ultraviolet light - Wear protective goggles, do not look directly at the emitted light
High voltage - Disconnect unit before servicing
Do not set the remote on clothing or bedding, emitted light may cause a fire.

6.3 Crime-lites 80S and 80L

The Foster + Freeman Crime-Lite 80S is a high intensity forensic light source comprised of light emitting diodes (LED) and associated beam conditioning lenses. BCI uses the blue/green light with a wavelength of 460-510 nm.

The Crime-Lite 80L is a linear, high intensity white light source that delivers broad, low angle illumination to assist in the examination of large items for biological stains. The 80L utilizes colored filter strips that attach magnetically to the unit and provide contrasting wavelengths that can be used to locate biological stains.

Both the 80S and 80L units operate on either a DC Power unit or a rechargeable battery. The DC Power unit plugs into an outlet and directly into the back of both the 80S and 80L units.

CAUTION: Switch off the 80S or 80L units before connecting the adaptor.
Read the instructions supplied with the battery charger before charging a battery. Failure to do so may result in shortening the life of the battery.

6.4 Crime-lite 82 Pro

The Crime-lite 82S Pro is a hand-held powerful upgrade to the other Crime-lite sources. It uses 16 high-intensity LEDs with the blue wavelength range of 420-470 nm.

6.5 Crime-lite ML and ML2

The Crime-lite ML/ML 2 combines the versatility of multi-wavelength, high intensity LED illumination and the comfort of wide area bi-ocular magnification to provide an effective bench top search and examination tool for the forensic scientist. BCI uses the violet light with a wavelength of 395-425 nm and the blue light with a wavelength of 430-470 nm.

6.6 Crime-lite ML2 for IR Photography

The Crime-lite ML2 has an IR camera to visualize and document images. Four LEDs with an output waveband of 800-900nm are used to illuminate blood with infrared.

6.7 Fujifilm HS-30 EXR Digital Camera for IR Photography

The Fujifilm HS-30 EXR Digital camera has the internal IR blocker removed and uses an attachable Infrared-passing filter that allows IR light to pass through but blocks most of the visible light spectrum. BCI utilizes a PECA 914 (89B) IR filter that can detect wavelengths greater than 700nm. A tungsten light may be utilized in conjunction with the camera to provide a greater amount of IR light.

6.8 Operation of Crime-lites

Place the item to be examined on an unused sheet of brown paper on a clean laboratory bench in a room that can be darkened. Use the 80S/80L/ML/ML 2 units plugged into either the DC Power unit or the battery holder and orange colored goggles/filters. Larger items such as sheets, comforters, blankets, etc. can be laid out on a large table or hung over metal racks and processed with the 80L or ML/ML 2 units.

The 80L unit can be used without a filter for the examination of dark clothing and bedding for the presence of minute bloodstains.

Where no potential body fluid stains are detected by visual examination and no chemical body fluid testing is performed: No stains for analysis.

Where chemical body fluid testing is performed but clarity is needed regarding other fluids of interest, use the wording for the fluid tested as well as:

No additional stains for analysis

Ex.: For ALS θ, TMB ⊕ results, use:
Presumptive positive for blood.
No additional stains for analysis.

6.9 Operation of IR Camera

Place the item to be examined on an unused sheet of brown paper on a clean laboratory bench. Ensure enough IR-emitting light is available (daylight or use of a tungsten bulb). Set the camera to automatic mode, using either manual or auto focus. Set the Film Simulation to Black & White. The IR function of the Crime-Lite ML2 can also be used.

Use the camera window to look for any areas on the item where IR light is being absorbed and appears darker than the surrounding area. Mark any areas that remain dark for possible areas to test for blood.

The IR camera is a tool and does not guarantee the visualization of every blood stain. Different background materials may not be as conducive to reflecting IR light.

Where no potential bloodstains are detected by visual examination and/or IR camera and no chemical testing is performed: No stains for analysis.

6.10 References

1. Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, National Institute of Justice, Washington, D. C., 1983, p. 178.
2. Kobus H, Silenieks E, Scharnberg J, Improving the effectiveness of fluorescence for the detection of semen stains on fabrics, J Forensic Sci, 47(4), 2002, pp. 819-823.
3. http://www.fosterfreeman.sinstr.ru/buklet/CL_ML.pdf
4. http://www.fosterfreeman.com/product/crime-lites/336-crime-lite-ml2.html

7 Wurster/Laux Paper for Saliva

7.1 Introduction

The Wurster/Laux (W/L) test is a rapid, non-quantitative presumptive test used to identify the presence of possible saliva stains. It is based on the ability of amylase, a component of saliva and other body fluids, to hydrolyze starch.

7.2 Safety Considerations

Iodine – Irritant

7.3 Preparations

Iodine Stock Solution – purchased 0.1N Iodine

Amylase Diffusion Buffer (PBS pH 6.9) Dissolve premix two containers of amylase diffusion buffer (SERI) in 1000 ml of Molecular Biology Grade water.

Starch Solution			While stirring, add the starch to the amylase diffusion buffer in a flask and bring to a boil on a stir plate. When all of the starch granules are in solution, allow the solution to cool to a warm temperature.
Amylase diffusion buffer	1000	Ml	
Soluble starch	1.0	G	

Wurster/Laux Paper

1. Cut sheets of thick chromatography paper (Fisher 05-714-4) into desired sizes.
2. Pour warm starch solution into a large container.
3. One at a time, place the filter paper pieces into the container and saturate the paper with the starch solution.
4. Place the saturated filter paper on sheets of aluminum foil and allow to dry. Aluminum foil may be tented over paper if necessary to protect paper while drying.
5. Wrap the paper in aluminum foil, then a manila envelope, then a sealed plastic bag to avoid moisture and store in the freezer.

Low Level Amylase Standard				Add the water directly to the stock bottle and vortex. Store in freezer as Stock Solution aliquots. Working Standard can be prepared by mixing 160 µl Stock Solution with 2 ml Molecular Biology Grade water. Aliquot 20 µl into 200 µl PCR tubes. Store tubes frozen in a pipette tip
Stock Solution	α-Amylase (from human saliva) (Sigma A1031)	5000	units	

	Molecular Biology Grade water	10	mL	rack. To use, thaw a tube and add the aliquot to a designated area of the W/L paper. This positive control equals 0.8 U/20µl.
Working Solution	Stock solution	160	µL	
	Molecular Biology Grade water	2	mL	

7.4 Procedure

1. Lightly moisten the W/L paper with Molecular Biology Grade water and place it over the suspected stain.
2. Press evenly with weigh paper between the paper and gloves for approximately 10-15 seconds.
3. Remove the W/L paper and place it on a watch glass, in a weigh boat, ~~or other container.~~ or other non-absorbent surface. Add the amylase standard to a designated labeled area on the paper. Document the preparation date for the paper and note the result of the controls using (+) in the case notes.
4. Incubate the W/L paper for approximately 10 minutes at room temperature.
5. Gently add a ~1:100 dilution of Iodine Stock solution (amber color) over the W/L paper. Do not rinse. The dilution may be increased if the diluted iodine solution appears too dark.
6. Photograph positive results from maps with the paper next to the item.

7.5 Interpretation of Results

The positive control must be positive and documented. A white area on a blue background indicates a positive result for amylase. A $\oplus$ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. Results that are unable to be documented in a photograph are reported as negative for amylase. Refer to the reporting section for report wording.

If the positive reaction is speckled in appearance, the following comment may be used in the Remarks section of the report or may be included as a table footnote:

The positive amylase area is similar to a mist or spatter pattern. Coughing and sneezing cannot be ruled out as possible origins of the stain.

7.6 References

1. Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology, and Biochemistry, National Institute of Justice, Washington, D. C., 1983, pp. 183-189.
2. Wurster, J.W. and Laux, D.L. 1990. A rapid amylase mapping procedure. MAFS Newsletter 19:48-49.

8 Acid Phosphatase for Semen

8.1 Introduction

The acid phosphatase (AP) test is an enzymatic reaction. It is a qualitative assay and uses alpha-naphthyl phosphate as the substrate. The acid phosphatase test is a preliminary color test and is only a presumptive test for semen. It is not a confirmatory test since acid phosphatase is found in other substances, including other body fluids.

8.2 Safety Considerations

Sodium α-naphthyl phosphate- Irritant
Brilliant Blue B (Ortho-Dianisidine) - Carcinogen
Weigh out reagent in a hood.

8.3 Preparations

AP reagent from SERI			
AP spot test premix reagent (PMR)	0.26	g	Mix the acid phosphatase spot test PMR and Molecular Biology Grade water. The AP reagent should be stored in a dark bottle and refrigerated when not in use.
Molecular Biology Grade water	10	mL	

Low Level AP Standard			
Acid phosphatase (from potato) Sigma P-1146	50	units	Add water directly to a freshly opened bottle of acid phosphatase and vortex gently. Add the solution to an ~8cm x 10cm piece of Fisher 05-714-4 chromatography paper and allow to dry. Cut the paper into ~3mm x 3mm squares (makes approximately 860 squares at a concentration of 20 units acid phosphatase/mL). Store in the freezer. To use, remove a test square from the freezer, place onto a small piece of filter paper and add 1 drop of the AP reagent. If a purple color develops within 10 minutes, the reagent can be used. A neat semen stain is equivalent to ~250 units acid phosphatase/mL. The standard equates to a ~1/10 dilution of semen.
Molecular Biology Grade water	2400	µL	

8.4 Procedure

1. Each day, before using the AP reagent on evidence, test it with the known acid phosphatase standard and negative control consisting of a blank swab or filter paper. If the acid phosphatase standard gives a positive result and the negative control gives a negative result the reagent may be used. Document in the case notes the lot number and note the result of the controls using (+) or (-). The initials of the analyst that performed the QC check must be on the daily QC check form.
2. Moisten a swab or filter paper with Molecular Biology Grade water and rub or blot (“map”) over the stain.
3. Add the AP reagent and look for a color change to purple/pink.
4. Results can be read at 10 minutes. Positive results may be noted prior.

5. Photograph positive results from maps.

8.5 Interpretation of Results

A purple/pink color change is a positive result. A ⊕ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. If case facts and circumstances indicate the potential for a false negative, a second semen test may be performed at the discretion of the forensic scientist. Refer to the reporting section for report wording. Older semen stains may yield an AP negative result, so samples may still be forwarded for DNA testing.

8.6 References

1. Metropolitan Police Forensic Science Laboratory Biology Methods Manual, 3-17 to 3-19, London, England (1978).
2. Schiff, A.F. 1978. Reliability of the acid phosphatase test for the identification of seminal fluid. JFS 23:853-844.
3. Laux, Dale L. 2003. Forensic Detection of Semen I: The acid phosphatase test, Midwestern Association of Forensic Scientists Newsletter 2:6-10.

9 Kernechtrot-Picroindigocarmine Stain for Spermatozoa

9.1 Introduction

The presence of semen in questioned specimens can be confirmed by the observation of one or more spermatozoa. A biological stain such as Nuclear Fast Red (Kernechtrot)-Picroindigocarmine stain, also known as the Christmas Tree stain, may be used on slides to enhance the appearance of the spermatozoa. With this stain, nuclear material stains red and background materials, including cellular material, stain green. The spermatozoa head stains red with the acrosomal cap remaining clear, while the tail (if present) stains green. Spermatozoa should be identified with a 10x objective or greater on the compound microscope.

9.2 Safety Considerations

Aluminum sulfate – Severe irritant
Indigocarmine – Slightly toxic; irritant
Ethanol – irritant
Picric acid- Severe Irritant; explosive
Permount – Flammable; irritant
Nuclear Fast Red – Slightly toxic; irritant

9.3 Preparations

Nuclear Fast Red Stain (Kernechtrot)			Dissolve the aluminum sulfate in the hot Molecular Biology Grade water and add the nuclear fast red. Stir the solution overnight, allow it to cool, and then filter. Store refrigerated.
Aluminum sulfate	5	g	
Hot Molecular Biology Grade water	100	mL	
Nuclear fast red	0.1	g	

Saturated Picric Acid **Do not let the stock dry out**
Prepare by adding Molecular Biology Grade water directly to the bottle of reagent, stir, let settle, and decant as needed. Saturated picric acid solution may be purchased.

Picroindigocarmine Stain (PICS)			Combine the indigocarmine and saturated picric acid. Stir overnight, and then filter. Store in the refrigerator.
Indigocarmine	0.33	g	
Saturated Picric Acid	100	mL	

Note: Nuclear Fast Red and Picroindigocarmine Stain can be obtained pre-prepared from SERI

9.4 Instrumentation

A compound microscope with 10x, 20x and 40x objectives is required

9.5 Procedure

9.5.1 Method 1

1. Use a previously prepared slide (from SAEC kit) or prepare a slide from an extract.

a. Extracts may be made from cuttings of swabs or fabric placed into a tube. For swabs, cut approximately 1/8-1/16 of each swab supplied, regardless of AP result. For fabric, cut 5 mm x 5 mm samples, if the AP reaction was weak, consider cutting 10 mm x 10 mm.
b. Add between ~50-100 µl (to cover), depending on the sample, of water.
c. Vortex the extract for 20 seconds and let the sample sit for 5 minutes.
d. Vortex the extract again for 20 seconds and spin briefly to remove droplets from the top and sides of the tube.
e. Remove 15 µl of the extract to a microscope slide. A grease pencil may be used to circle the stain.
f. Air dry the slide to fix the sperm. A heat block may be used to speed drying. ~~A grease pencil may be used to circle the stain.~~

2. Add enough Nuclear Fast Red stain to cover the sample. A clean wooden stick may be used to spread out the stain. The stain should stay on the slide for at least 15 minutes, but not until the stain dries. Rinse the stain off with Molecular Biology Grade water.
3. Add enough PICS stain to cover the sample. Allow the stain to stay on the slide for approximately 10 – 15 seconds. Rinse the stain off with reagent grade ethanol. Caution: Over staining may occur if the stain is left on for more than 20 seconds.
4. Allow the slide to air dry or use a heat block, then add a small amount of Permount or Cytoseal mounting medium and apply a cover slip. Using a microscope, observe the slide with a 10x objective or greater and rate using the ++++ system. In the case notes, rate and record each examined slide. For slides that receive a rating of few or single, record in the case notes the serial number or other unique identifier of the microscope used and coordinates for four sperm cells, if possible. A second forensic scientist that is currently proficient in biology must confirm a "few" or single sperm cell rating by independently documenting the results of the verification, initialing and dating in the notes. If the verification is not consistent with the original observation, the final resolved rating will be clearly recorded in addition to the initial observations. Document reagents used on the reagent log.
5. The following are the guidelines of the ++++ rating system:

4+	Many sperm cells in every field.
3+	Many or some sperm cells in most fields.
2+	Some sperm cells in some fields, easy to find.
1+	Some sperm cells in some fields, hard to find.
Few	Small numbers of sperm cells on the entire slide.
Single sperm cell	One sperm cell on the entire slide.
θ	No sperm cells present on slide.

9.5.2 Optional method

1. If a swab is used to test the AP reaction of a stain and the reaction is strong, the extraction procedure may be skipped and a slide prepared by pressing the swab onto a microscope slide while still damp.
2. Prepare and examine a slide as in 9.5.1 beginning at step 1f.
3. If no sperm cells are identified, make an extract (see 9.5.1).

9.6 Interpretation of Results

Human spermatozoa are stained red, oval, contain an acrosome, and are smaller than the nuclei of epithelial cells. Tails, if present, will stain green and should be noted with a "T" in the notes.

Some cells may resemble a sperm cell without having all the characteristics of one. This cell can be referred to as a QNC (Questionable, Not Called). This should be documented in the case notes when no sperm are observed with reasoning why the cell was QNC. A second forensic scientist that is currently proficient in biology should confirm by initialing and dating the QNC they have observed and also documenting as "QNC". A QNC result will be reported as inconclusive for semen unless seminal fluid is detected in further testing. If the hospital smears have not been examined and the analyst prepared slide has sparse cells or only QNCs, the corresponding smear should be stained and evaluated.

Refer to the reporting section for report wording.

9.7 References

1. Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology, and Biochemistry U.S. Government Printing Office: Washington, DC, 1983, pp. 149-154.
2. Federal Bureau of Investigation, Proceedings of a Forensic Science Symposium on the Analysis of Sexual Assault Evidence, U. S. Government.
3. Stone, I.C., Staining of Spermatozoa with Kernechtrot and Picroindigocarmine for microscopic identification, SWIFS Criminal Investigation Laboratory, September 1972.

10 Seratec PSA Semiquant Kit

10.1 Introduction

Prostatic specific antigen (PSA or p30), is a protein that was isolated and characterized by Sensabaugh in 1978. Antisera to the protein quickly became utilized in the forensic field for the detection of semen. The Seratec PSA Semiquant is an immunochromatographic one-step test for the detection of PSA. A 120 µl volume is added to the chamber that equates to a detection limit of 0.4 to 0.8 ng PSA. Although a strong PSA test result is highly indicative of semen, recent publications have documented the presence of PSA in body fluids other than semen. Positive PSA test results have also been obtained from lubricants and/or spermicides. This test is considered to be a presumptive test for semen.

10.2 Positive Reactions for PSA with the Seratec Kit

During the test, PSA from the fluid reacts with the gold labeled monoclonal anti-PSA-antibody and forms a PSA-gold labeled anti-PSA-antibody-complex. Through the capillary effect of the membrane, the reaction mixture including the complex is carried across with the fluid. Upon positive findings, the PSA-gold labeled anti-PSA-antibody-complex binds to the fixed monoclonal mouse anti-PSA-antibody on the membrane by creating a red line at the test region. Both monoclonal anti-PSA-antibodies detect different epitopes of the PSA. The intensity of the red lines depends on the concentration of PSA.
The colored gold labeled anti-PSA-antibody binds to the anti-mouse-antibody at the control region and region of the internal standard by developing a red line in each region. The two lines are independent of the existence of PSA in the fluid. The internal standard line has been adjusted to a color intensity of 4 ng PSA/ml.

10.3 Safety Considerations

No known safety hazards.

10.4 Procedure

1. Use the smallest possible amount of the stain (e.g. 0.25 x 0.25 cm or ~1/8 each swab). Extract the sample for at least 2 hours in 1.0 ml of the PSA Buffer provided with the Seratec Kit.
2. Centrifuge at 6000-8000 rpm for 3 minutes.
3. Add 120 µl of the supernatant to the sample well.
4. Read the results at 10 minutes. Photograph positive results at exactly 10 minutes. Write the case number, item number, and initials on the test kit (or document in the photograph) and the lot number, positive control result, and date performed in the case notes.

10.5 Interpretation of Results

- A pink line in the test, control area, and internal standard area is a positive result.
- A faint pink line *in the test area* that cannot be reproduced in the photo or copy is reported as negative. No filter enhancements are to be performed for documentation.
- A pink line in the control and internal standard areas and the absence of a pink line in the test area is a negative result, provided there is no high dose hook effect. [High dose hook effect occurs at concentrations above 500 µg PSA/ml. If this is suspected, dilute the sample and re-test].

- Any test without a control line and/or internal standard line is considered invalid and should be repeated.
- Refer to the reporting section for report wording.

10.6 References

1. Hochmeister M.N., et al., Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid, J Forensic Sci 1999 Sep;44(5):1057-60.
2. Laux, Dale L., Tambasco, Anthony and Benzinger, Elizabeth A. Forensic Detection of Semen II: Comparison of the Abacus Diagnostics OneStep ABAcard p30 Test and the Seratec PSA Semiquant Kit for the Determination of the Presence of Semen in Forensic Cases, Midwestern Association of Forensic Scientists Newsletter, Vol. 32, Fall 2003, pp. 11-18.
3. Laux, Dale L. and Custis, Sarah E. Forensic Detection of Semen III: Detection of PSA Using Membrane Based Tests: Sensitivity Issues with Regards to the Presence of PSA in Other Body Fluids, Midwestern Association of Forensic Scientists Newsletter, Vol. 33, Winter 2004, pp. 33-39.
4. http://www.seratec.com/docs/user_instructions/psa_in_body_fluids (accessed on 03/21/13)
5. http://www2.cedarcrest.edu/academic/bio/hale/biostatsummaries/haubois.html (accessed on 03/21/13)
6. Bitner, S.E., False Positives Observed on the Seratec PSA SemiQuant Cassette Test with Condom Lubricants, J Forensic Sci 2012 Nov;57(6):1545-48.
7. Snead, M.C., et al., Effect of topical vaginal products on the detection of prostate-specific antigen, a biomarker of semen exposure, using ABAcards, Contraception, In Press.

11 Creatinine for Urine (Jaffe Test)

11.1 Introduction

Creatinine is present in body fluids such as serum, saliva and sweat, but it is one hundred times more concentrated in urine than in other body fluids. The Jaffe test is used to reveal the presence of creatinine. *This is a presumptive test for urine*.

When picric acid is added to creatinine in the presence of NaOH, creatinine picrate forms an orange-colored product confirming the presence of creatinine, a component of urine.

11.2 Safety Considerations

Picric Acid – Severe irritant; explosive
Sodium Hydroxide – Corrosive, hygroscopic; causes eye and skin burns; causes digestive and respiratory burns

11.3 Preparations

1.25 M Sodium Hydroxide (5% Sodium Hydroxide)			
4.0 M Sodium hydroxide	31	mL	
Molecular Biology Grade water	69	mL	Store at room temperature

Saturated Picric Acid
Add Molecular Biology Grade water directly to the picric acid reagent bottle and stir. Allow the solution to settle prior to use. Store at room temperature.

Creatinine Standard			
Creatinine, anhydrous, Sigma C4255	0.1	g	Add 100 ml Molecular Biology Grade water to 0.1 g creatinine in a small beaker and stir until dissolved. Pour the solution into a 2L beaker and add cotton-tipped swabs. Soak swabs to saturation and dry. Store frozen. Prepares several hundred swabs (1.0 mg creatinine/ml). This equates to undiluted urine. To use, add a drop of 5% NaOH followed by a drop of concentrated picric acid. If an orange color develops within 5 minutes, the reagent can be used.
Molecular Biology Grade water	100	mL	

11.4 Procedure

1. Before using the picric acid and sodium hydroxide on evidence, test with the known creatinine standard and Molecular Biology Grade water. If the known creatinine standard gives a positive result and the Molecular Biology Grade water gives a negative result the reagents may be used. Document the result of the controls, prep date, and initials/lot numbers in the case notes.
2. Swab, map or cut a portion of the suspected stain area (~2-3 cm^2). Cut so that the stain covers about ½ of the cutting and the remaining portion is unstained. Alternatively, a separate sample from a non-stained area may also be used but there must be a substrate control. If the sample is still in liquid form, add a couple drops to a clean weigh boat.

3. Place the cutting on a piece of Whatman filter paper and add 1-3 drops of 5 % NaOH to the cutting at the junction of the stained and unstained areas. Alternatively, extract cuttings of the stained and unstained areas in test tubes and add the picric acid to the tubes.
4. Add 1-3 drops of saturated picric acid solution and view results after 5 minutes.
5. If a swabbing of the stain is negative, repeat the analysis on a cutting from the item.

11.5 Interpretation of Results

An orange coloration on the stained area and no orange coloration on the unstained area of a sample is a positive result for the presence of creatinine. A ⊕ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. Refer to the reporting section for report wording. A second substrate control may be used if the first is positive. A positive sample requires the result be observed in the photograph. Substrate control cutting must be shown in the photograph or detailed documentation in notes.

11.6 References

1. Gaensslen, R.E., Sourcebook in Forensic Serology, Immunology and Biochemistry, National Institute of Justice, 1983, pp.191-195.

12 Urobilinogen for Feces

12.1 Introduction

Tests for urobilinogen are used to indicate the presence of feces and are considered presumptive because urobilinogen can be found in other body fluids. Fecal material consists of water and solid waste materials made up of mucus, undigested food residue, and microorganisms. Fecal material is usually brown in color due to urobilinogen that is formed from bilirubin by reduction processes in the intestine. Fecal material may also be green, black, or red depending on diet, drugs, and pathological conditions. There is no confirmatory test for feces.

12.2 Safety Considerations

Zinc Chloride – Irritant; moderately toxic
Ethanol – Irritant

12.3 Preparations

Zinc Chloride Solution

1. In a small beaker, add enough zinc chloride ($ZnCl_2$) to EtOH to prepare a saturated solution (e.g., 1 g $ZnCl_2$ to 10 ml EtOH).
2. Allow the solution to settle prior to use.
3. Store the solution at room temperature.

12.4 Procedure

1. A positive control feces sample and an ethanol negative control will be run when testing evidence samples for urobilinogen. The positive and negative controls may be performed concurrently with the testing of evidence samples unless the evidence sample will be consumed in testing. For samples that will be consumed, the $ZnCl_2$ reagent must be tested with a known feces sample and ethanol prior to testing of the evidence sample. Additional positive and negative samples will be run with the consumed sample. Document the results of the controls, lot number, prep date, and initials in the case notes.
2. Place the sample in a test tube (either a cutting or a swab collected with visible staining on the swab, no swab stick will be in the tube) labeled with the case/item number and add enough ethanol to cover. Allow to extract in the ethanol for at least 30 minutes.
3. Add an equivalent amount of $ZnCl_2$ and gently shake.
4. Remove the cutting/swab from the test tube and examine the liquid with the Spex Crimescope CS-16, the Crime-lites 80S and 80L, Crime-lite ML, or Crime-lite ML2 and yellow barrier filter glasses. Document the results by photographing the sample and controls. Hold the samples against a dark surface to photograph.

12.5 Interpretation of Results

Use either a yellow shield to evaluate samples with the 430-470 nM wavelength filter or an orange shield with the 460-510 nM wavelength filter. An apple green fluorescence occurs if urobilinogen is

present. A ⊕ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. A positive sample requires the result be observed in the photograph. Refer to the reporting section for report wording.

12.6 References

1. Lloyd, J.B.F. and Weston, N.T., "A Spectrometric Study of the Fluorescence Detection of Fecal Urobilinoids", Journal of Forensic Sciences, Vol. 27, No. 2, April 1982, pp. 352-365.

13 Tetramethylbenzidine for Blood

13.1 Introduction

Tetramethylbenzidine (TMB) is a presumptive chemical color test for blood that is based on the peroxidase-like activity of the heme portion of hemoglobin. The IR camera may be helpful in identifying potential blood stains on dark items.

13.2 Safety Considerations

3, 3', 5, 5'-tetramethylbenzidine – Irritant
Ethanol – Irritant
Hydrogen peroxide - Eye irritant

13.3 Preparations

TMB reagent			Mix the TMB with the ethanol, and then add the glacial acetic acid. Store in a dark colored bottle in the refrigerator. Expires after 12 months unless a component expires sooner.
3,3', 5,5'- tetra-methylbenzidine	0.5	g	
Ethanol	350	ml	
Glacial acetic acid	3	ml	

3% hydrogen peroxide (H_2O_2) May be purchased from drug store. Store refrigerated.

Low Level Hemoglobin Standard (0.05 mg hemoglobin/ml)			**Stock:** Add 100 ml Molecular Biology Grade water to a bottle of hemoglobin and gently vortex. Place the solution in a graduated cylinder and bring to 1 L with Molecular Biology Grade water. Store frozen. **Working solution:** Prepare a 1:20 dilution (10 ml stock + 190 ml Molecular Biology Grade water) in a 2 L beaker and add cotton-tipped swabs (~300). Soak swabs to saturation and dry. Repeat. Store frozen. This equates to a 1 in 3000 dilution of blood. **To use:** Add a drop of TMB followed by a drop of 3% H_2O_2. If a blue-green color develops within 10 seconds, the reagent can be used.
Hemoglobin, human (Sigma H7379)	1	g	
Molecular Biology Grade water	1	L	

13.4 Procedure

1. Before using the TMB reagent and H_2O_2 on evidence, test them with the hemoglobin standard and negative control consisting of a blank swab or filter paper. If the hemoglobin standard gives a positive result and the negative control gives a negative result, the TMB reagent and H_2O_2 may be used. Document in the case notes the lot number and note the result of the controls using (+) or (-). The initials of the analyst that performed the QC check must be on the daily QC check form.
2. Small stains should be photographed before testing.
3. Moisten a swab or filter paper with Molecular Biology Grade water and rub or blot the stain. A dry swab may be rubbed against a substantial stain. The method used should be documented in the notes.
4. Add a few drops of the TMB reagent. Wait a few seconds while carefully observing to ensure no color change develops at this time.
5. Add a few drops of the 3% H_2O_2 and wait up to 10 seconds for a color change.

13.5 Interpretation of Results

A rapid greenish-blue color change after addition of the H_2O_2 is a positive result. If the swab or filter paper does not turn greenish-blue within 10 seconds, it is a negative result. A $\oplus$ or (+) may be used to indicate a positive result and a θ or (-) may be used to indicate a negative result. If there is a color change after adding the TMB reagent but prior to the H_2O_2, the result is inconclusive for blood and additional testing should be performed if not consuming sample. Refer to the reporting section for report wording.

13.6 References

1. Holland, V.R., et. al. 1974. A substitute for benzidine in the detection of blood. Tetrahedron 30:3299.
2. Gasner, D.D., et. al. 1976. An evaluation of tetramethylbenzidine as a presumptive test for blood JFS 21:816.
3. Tetramethylbenzidine: a reported noncarcinogenic analog of benzidine. 1989. Aldrichimica Acta 22:48.
4. Cox, M. 1991. A study of the sensitivity and specificity of four presumptive tests for blood. J. Forensic Sci. 36:1503-1511.

14 HemDirect for Human Blood

14.1 Introduction

Hemoglobin (Hb) is located in the erythrocytes and predominantly serves the transport of oxygen and carbon dioxide within the body. The HemDirect test is an immunochromatographic one-step test for the detection of human blood. This test is considered to be confirmatory for human blood.

14.2 Positive Reactions with the HemDirect test

If human hemoglobin is present in a sample, the human hemoglobin (hHb) reacts with a mobile monoclonal antihuman hemoglobin antibody forming a mobile antigen-antibody complex. This antigen-antibody complex migrates through the absorbent device toward the test area. In the test area there is an immobilized monoclonal anti-human hemoglobin antibody. This immobilized antibody captures the above complex resulting in an antibody-antigen-antibody complex. If the human hemoglobin concentration is greater than 0.05 µg / ml the pink dye particles form a pink colored band in the test area, indicating a positive result. The HemDirect test incorporates an internal positive control consisting of gold labeled rabbit antibodies that are captured by an immobilized polyclonal goat anti-rabbit antibody present in the control area, forming a complex. High dose hook effect is a false negative that occurs if there is more hHb than can be bound by the mobile monoclonal anti-human hemoglobin antibody. The excess hHb binds to the immobilized polyclonal anti-human hemoglobin antibody, thus blocking binding to the mobile antigen-antibody-dye complex in the test area. This test cross-reacts with ferret and higher primate blood.

14.3 Safety Considerations

No known safety hazards.

14.4 Preparations

Make sure that the sample is at room temperature prior to analysis.

14.5 Procedure

1. Use the smallest possible sample (e.g. ~5x5 mm or ~1/8 swab) depending on the intensity of the stain.
2. Allow the sample to extract for approximately 30 minutes in the buffer tube supplied with the kit. Overnight extraction may help older samples. If the extract is cloudy or contains debris, centrifuge to clarify.
3. Add ~150 µL (three drops from the buffer tube) of the extract to the sample well, of the HemDirect test. Record both buffer and card lot numbers and date performed in notes.
4. Read the results at 10 minutes. Photograph positive or inconclusive results or copy the results at exactly 10 minutes. Write the case number, item number, and initials on the test cassette (or document in the photograph). Document that the positive control worked.

14.6 Interpretation of Results

1. A pink line in both the test and control areas is a positive result.
2. A faint pink line that cannot be reproduced in the photo or copy is reported as no human blood identified.
3. A pink line in the control area and the absence of a pink line in the test area is a negative result, provided there is no high dose hook effect. [If this is suspected, dilute the sample and re-test]
4. Any test without a control line is considered invalid and should be repeated.

5. Refer to the reporting section for report wording.

14.7 References

1. Hochmeister, M., Rudin O., Sparkes, R., Gehrig, C., Schmidt, L. Evaluation of an Immunochromatographic 1-Step Blood Test for the Forensic Identification of Human Blood. J. For. Sciences May 44(3), 597-602, 1999.
2. Misencik, A., and Laux, D.L. Validation Study of the Seratec HemDirect Hemoglobin Assay for the Forensic Identification of Human Blood, MAFS 2005 Fall Meeting, Oct. 7, 2005, St. Louis, Missouri
3. Spear, T. F., Binkley, S. A. The HemeSelect test: a simple and sensitive forensic species test. J. For. Sci. Soc.34 (1), p 41-6, 1994.
4. Fernando, S.A., Wilson, G.S. Studies of the 'hook' effect in the one-step sandwich immunoassay. J. Imm. Methods 151 (1-2), pp. 47-66, 1992.

15 Hair Examination

15.1 Introduction

Cases such as hit skips, assaults, and homicides may be submitted requesting hair examination. If no body fluid is detected, the analyst may examine the trace evidence with the objective to identify clearly relevant apparent hairs with a root for DNA testing. A microscope may be used to assist in the identification of a root. Hair evidence submitted in a sexual assault kit is routinely examined for the presence of body fluids. No hair comparisons will be performed.

15.2 Procedure

15.2.1 Examination of non sexual assault kit evidence

If a body fluid suitable for DNA testing is detected in the case and a clearly relevant apparent hair is observed, then the apparent hair is retained for potential future analysis as follows.

- A single/few apparent hair(s) are observed:
  - Attach the apparent hair to a Post-It™ note
  - Place the Post-It™ note in a coin envelope
  - Re-package the coin envelope within the original evidence item package.
  - Document in the notes
- Many apparent hairs are observed:
  - Leave the hairs on the evidence item and document in the notes
  - The examination paper should be folded and packaged with the original evidence item package to prevent loss.

Should the DNA testing not yield probative results, a request may be made by the DNA analyst to have the apparent hair analyzed to determine if a root is present for DNA testing. If a root is present, cut off approximately a 1 cm section or less containing the root and place in a tube. Retain the remainder of the hair with the parent item in a coin envelope.

15.2.2 Examination of sexual assault kit evidence

An ALS or stereoscope may be used to examine apparent hairs for potential semen stains. If body fluids are indicated, use a single SEB swab to collect a sample for possible DNA testing. If additional screening procedures will be performed, samples may be collected with a single swab with water.

If trace debris is observed on a swab, try to remove it prior to screening the swab and report the trace debris. If the debris cannot be removed from the swab, then screen the swab and list the debris in the report with the sample on the same line.

For DNA testing, cut off approximately a 1 cm section or less containing the root and place in a tube. Retain the remainder of the hair with the parent item in a coin envelope.

15.3 Documentation

1. When the objective is to determine the source of DNA from a hair, case notes should include if a root is present.
2. Refer to report wording section.

DNA Methods

Changes and updates in the manual do not imply that previously issued methods and policies are incorrect or invalid.

16 DNA Isolation

16.1 Organic Extraction

16.1.1 Introduction

The isolation of DNA depends on the removal of proteins and other compounds from the DNA. This is accomplished through the use of SDS to denature the proteins and proteinase K, a serine proteinase, to cleave the proteins into smaller pieces. The DNA is then purified through a combination of phenol precipitation of the proteins and filtration to remove residual extraction reagents and contaminants.

16.1.2 Stopping Points and Storage

Once DNA extraction has begun, the process can be stopped at virtually any point. For overnight or over weekend pauses, ambient, refrigerator or freezer storage does not harm DNA. For long term storage, DNA solutions are generally frozen. Avoid setting up amplification reactions until shortly before thermal cycling.

A differential extraction is used for samples that may contain a mixture of spermatozoa and non-sperm cells. The initial extraction step releases non-sperm DNA while leaving the sperm cells intact. The sperm cells are then pelleted and washed prior to lysis.

Indicate in the case notes which extraction method is being used for each sample.

16.1.3 Differential Extraction of Seminal Stains

1. Cut out a portion of the stain or remove up to two very thin swab SHELLS from the applicator sticks and place into an extraction tube. If necessary, cut the fabric into two or three pieces so that it is covered by the stain extraction buffer.
2. Also prepare a manipulation blank at this time and label as **MB F1/F2 Date Initials**. This manipulation blank must be representative of the most concentrated sample. If samples in a batch may follow divergent analysis paths, use multiple blanks.
3. Add 500 µl Stain Extraction Buffer and 5 µl 20 mg/ml Proteinase K to each tube.
4. Mix well and incubate at least two hours at 37°C.
5. Make sure the tube is firmly closed and vortex at maximum speed for at least one minute. Spin briefly before opening the tubes. Put the sample cuttings in the basket and centrifuge at maximum speed for five minutes to spin out the extraction buffer and pellet the sperm cells. Discard spin basket and cutting unless otherwise noted below or on the manifest.
6. Carefully remove the majority of the supernatant in the original tube and place into a new extraction tube labeled "F1". This fraction should contain primarily the non-sperm cell lysate. Remove as much of the remaining supernatant as possible, being careful not to disturb the pellet.
7. The remaining cell pellet in the original tube should contain primarily the sperm cells from the sample. This tube is labeled "F2".
8. Rinse the pellet (F2) with approximately 1000 µl TNE, vortex briefly and centrifuge at maximum speed for 10 minutes. Remove as much of the TNE wash as possible without disturbing the

pellet. Repeat this process at least two more times. Discard the washes. If it is difficult to remove the majority of the supernatant, compensate by performing additional washes.

9. To the F2 add 350 µl SEB, 40 µl 390 mM DTT and 10 µl 20 mg/ml Proteinase K.
10. Mix and incubate at 37°C for at least two hours. Spin tubes briefly before opening.
11. Continue processing F1 and F2 fractions at the phenol step. (See step 5 below, phenol extraction).

16.1.4 Non-Semen Body Fluid Stains or Liquid Blood

1. Cut out a portion of the stain or remove the outer layer of the swab from the applicator stick and place into an extraction tube. If necessary, cut the fabric into smaller pieces so that it is covered by the stain extraction buffer. Also, prepare a manipulation blank at this time and label as **MB Date Initials**. If samples in a batch may follow divergent analysis paths, use multiple blanks. For liquid blood, add up to 50 µl of liquid blood to a microfuge tube and process as a stain. Any blood collection tube is acceptable, although red-top clot tubes do not have a preservative and are least desirable for prolonged ambient storage.
2. To each tube add 400 µl Stain Extraction Buffer, 10 µl 20 mg/ml Proteinase K and 5 µl 390 mM DTT. Mix and spin briefly to force the cutting into the liquid.
3. Incubate at 56°C several hours to overnight. Spin tube briefly before opening.
4. Place the cutting into a basket and place the basket into the tube. Spin 3 minutes at maximum speed to remove the stain extraction buffer from the cutting. Discard the spin basket and cutting unless otherwise noted below or on the manifest.

   For reference standards on cotton swabs or fabric and small cuttings from evidence samples, the substrate may remain in the tube during phenol extraction or it may be removed and discarded without spinning prior to phenol extraction.

5. Phenol Extraction: Add 500 µl phenol/chloroform/isoamyl alcohol. Do this step in the fume hood. Shake vigorously or vortex for several seconds to achieve a milky emulsion in the tube. Spin the tubes for two minutes at max speed.

   Do not use microfuge tubes provided for use with Microcon filters for organic extractions or high-speed centrifugation—they crack

6. Transfer the aqueous phase (top layer) to a labeled Microcon 100 tube. Spin at 500 x g for 8 to 10 minutes or until no liquid remains on the filter. A slightly faster speed may be used if the sample does not go through the filter. Add 50-100 µl of TE to the filter and centrifuge as before to wash residual extraction components from the DNA. Multiple washes may be performed. Examine the filter unit to verify that no tearing or cracking has occurred. Contact the Technical Leader if tearing or cracking is observed.
7. Add 50 or more µl TE (depending on anticipated DNA recovery). Consideration of the possible need for Y-STR amplifications, etc., should be made in determining the elution volume. Manipulation blanks must be analyzed at the same concentration conditions as required by the batch forensic sample containing the least amount of DNA.
8. Invert into a new, labeled tube and spin briefly for up to 1 minute. Dispose of the filter.
9. Record the quantity of TE used for recovery for each sample on the amplification set-up worksheet or batch manifest if different than 50 µl.

16.2 Maxwell RSC 48 Procedure

The isolation of DNA depends on the removal of proteins and other compounds from the DNA. This is accomplished through the use of salts to denature the proteins and proteinase K, a serine proteinase, to cleave the proteins into smaller pieces. The DNA is then purified by binding to magnetic resin and a series of washes to remove residual extraction reagents and contaminants.

Reagent Preparations

1. Proteinase K: Add 556 µl of nuclease-free water (comes in reagent kit) to the tube of proteinase K to make 18mg/mL solution and store in freezer.
2. Extraction Master Mix: The table below shows the amount of each component needed for a sample extraction. LF-DNA Maxwell Buffer Master Mix Prep is provided for making larger batches of the master mix.

Component	Volume (µl)
Casework Extraction Buffer	386
Proteinase K (18 mg/ml)	10
Molecular Biology Grade water OR Thioglycerol**	4
Total Reaction Volume	400
****The first differential incubation does NOT use thioglycerol**	

Non-Differential Samples

1. Place non-differential samples into slotted-bottom spin baskets and 2.0 ml tubes.
2. Prepare extraction buffer master mix WITH thioglycerol.
3. Pipette 400 µl directly into the slotted spin basket and close the tubes.
4. Vortex briefly but DO NOT SPIN.
5. Incubate at 56°C for approximately 30 min but no longer than overnight.
6. Vortex and spin for 3 or more minutes at max speed.
7. Discard the slotted spin basket with the cutting after verifying that all liquid is in the tube.

GO TO MAXWELL SETUP STEPS

→**Do not start setting up the cartridges manually, on the Tecan, or Maxprep unless there is time to reach the end of the protocol with the DNA in the elution tubes**←

First Differential Incubation

1. Place differential samples into 2.0 ml tubes without spin baskets.
2. Prepare an extraction buffer master mix using the Molecular Biology Grade water option.
3. Pipette 400 µl into each tube.
4. Vortex briefly.
5. Incubate at 56°C for approximately 30 min but no longer than overnight.

Differential Separation

1. Vortex 1 minute and spin briefly.
2. Remove cutting and place in an open grid spin basket.
3. Spin for 5 min at max speed.
4. Discard cutting and the open grid spin basket.
5. Remove majority of liquid to new tube labeled F1 while avoiding pellet. Set F1 tubes aside.
6. Add 1000 µl TNE to each F2 tube, mix briefly, and spin 5 min at max speed.
7. Remove liquid and discard.
8. Repeat wash (steps 6 and 7) 2 more times.

Second Differential Incubation

1. Prepare an extraction buffer master mix WITH thioglycerol.
2. Pipette 400 µl into each tube.
3. Vortex briefly.
4. Incubate at 56°C for approximately 30 min but no longer than overnight.

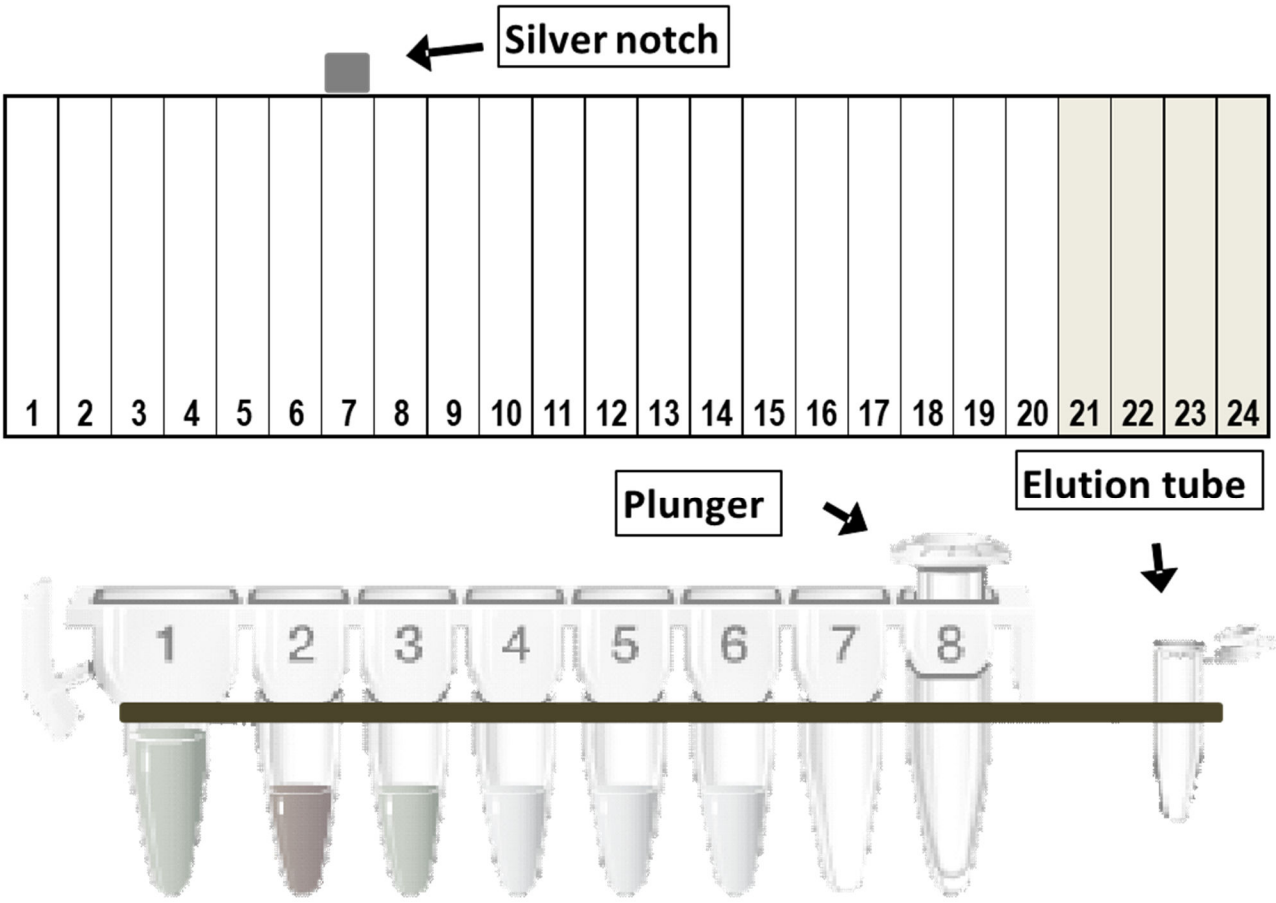
GO TO MAXWELL SETUP STEPS

→**Do not start setting up the cartridges manually, on the Tecan, or Maxprep unless there is time to reach the end of the protocol with the DNA in the elution tubes**←

Manual Maxwell Setup Use up to 24 positions if performing manual setup

1. Place one Maxwell cartridge into black Maxwell tray for each sample. Place cartridges in any space. Both ends will click into place.
2. Peel the protective cover off of each cartridge.
3. Place a *numbered* empty elution tube in the open slot with the open cap facing away from the cartridge.
4. Pipette 50 µl of elution buffer into the bottom of each elution tube.
5. Mix and spin samples briefly.
6. Pipette 200 µl of lysis buffer into each sample and then pipette the entire 600 µl into well 1 of each corresponding cartridge.

GO TO MAXWELL PROCESSING



Maxwell cartridge drawing and layout. Note position of plunger in well 8 and orientation of elution tube with cap facing away from cartridge. For Tecan automated setup, limit samples including blanks to 20 for each Maxwell tray. For Maxprep and manual setup, process up to 24 samples including blanks for each Maxwell tray.

***The silver notch will be on the right side of the back Maxwell tray near cartridge 40.**

Tecan Automated Maxwell Setup Limit samples to 20 per tray – Deck check must be done before Tecan processing begins.

1. Place required number of Maxwell cartridges into the front Maxwell tray beginning with position 1 and leaving no spaces between cartridges. Both ends will click into place. For the back Maxwell tray, begin at position 25.
2. Peel the protective cover off of each cartridge.
3. Place *numbered* elution tubes onto the Maxwell tray with caps facing away from cartridges. Ensure the elution tubes are seated all the way down into the tray.

4. Mix and spin pre-extracted samples briefly.
5. Ensure pre-extraction tube labels are consistent with manifests and load the pre-extraction tubes into the Tecan sample carrier.
6. Turn on the Tecan instrument and then open EVOware Standard software.
7. Choose “Run an existing script” or “Edit an existing script”.
8. Navigate to the “Combined_Maxwell_Setup” script.
9. Select “Run” through multiple prompts until the script begins.
10. Follow script prompts for the deck setup. Place black tray into custom Tecan carrier with silver notch facing the right side of the Tecan robot.
11. Enter number of samples being extracted when prompted.
12. Tecan EVO 150 will begin processing.
13. After script completes, select “cancel” to return to EVOware startup menu.
14. When Tecan has completed processing, discard reagents and remove pre-extracted sample tubes from deck.
15. Remove black Maxwell tray from custom carrier.
16. Check that all elution tubes have elution buffer at the bottom of the tube and none of the tubes have liquid along the sides.

GO TO MAXWELL PROCESSING

MaxPrep Automated Set-up ~~(added in DNA Methods manual revision 13)~~

Note: Do not update the date and time on the Maxprep computers. If this is done, it interferes with the instrument connection and Promega service is required.

Maxprep AS1550 DNA IQ – Tubes (Version 1.2.0/1) method pre-processing instructions.

1. Ensure the daily maintenance has been performed prior to starting a run. The weekly maintenance may be run in place of the daily maintenance at the start of the week.
2. Prepare the deck trays. *Numbered* microcentrifuge tubes for elution should be open with the Maxwell cartridges unsealed and placed in the appropriate locations on the deck tray. The opened tube caps must all be facing the left side of the Maxprep instrument when trays are properly seated in the instrument.
    - Do not leave gaps between cartridges.
    - Add cartridges starting with the position one on the front tray.
    - The front tray must be filled with 24 cartridges before starting the back tray.
3. Pre-processed samples will be placed on the deck of the MaxPrep Liquid Handler with QR barcodes *or labeled tubes and numbered 1-48 for the front and back trays (similar to the elution tube labeling system described below) with the manifest.* *If QR codes are used*, ensure that the QR code is clearly

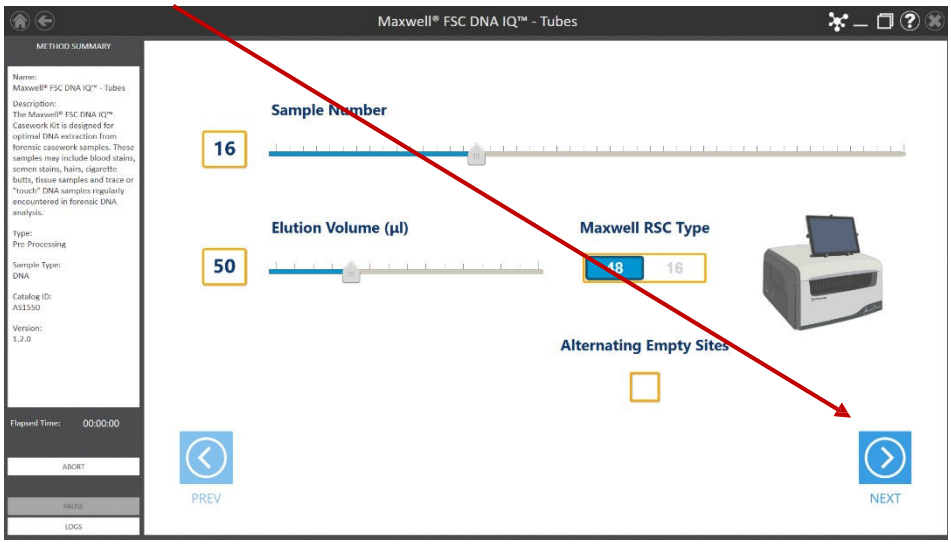
available in the tube rack for scanning by the instrument. All tubes *but the F1 tubes*, must be labeled with the case/item number or reagent blank identifier.

Tube rack labeling system – Elution tube caps should be labeled with the tube number correlating to the sample listed on the manifest. If more than one batch is being extracted at the same time, labeling should be adjusted accordingly to ensure that a 3rd party could easily identify and differentiate between tubes from one batch and tubes from the second batch.

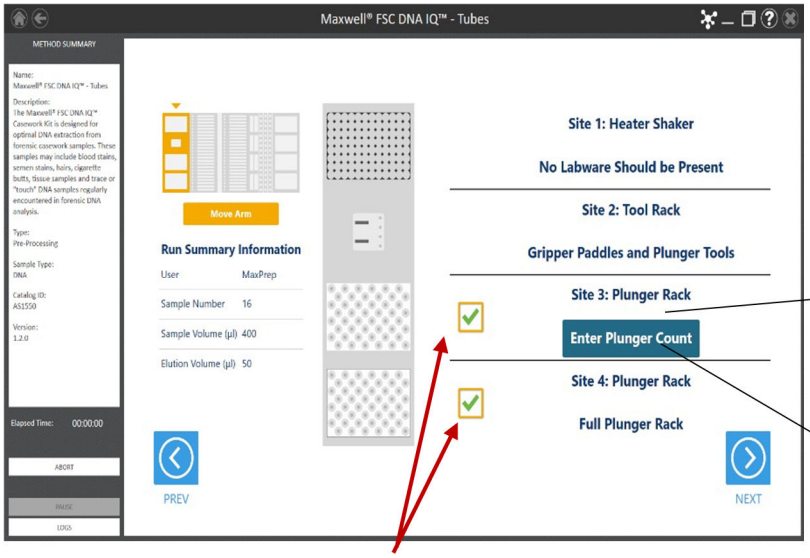
Tube checks vs manifest –
(*1) QR codes used -* After sample tubes have been pulled from biology for extraction, a QR coded tube label should be applied to each tube. F1 tubes will need to be prepared at this time as well. Initial tube checks should ensure that the correct tubes have been pulled from biology, that they are labeled properly with a QR label, and that they are in the correct order for extraction, according to the manifest.
(*2) Tube numbering system - After sample tubes have been pulled from biology for extraction, tubes will be labeled with the tube number correlating to the sample listed on the manifest (1-48). F1 tubes will need to be prepared at this time as well. If more than one batch is being extracted at the same time, labeling (25-48) will help ensure that a 3rd party could easily identify and differentiate between tubes from one batch manifest and tubes from the second batch manifest. Initial tube checks should ensure that the correct tubes have been pulled from biology, that they are labeled properly, and that they are in the correct order for extraction, according to the manifest. Maxprep tube rack 1 will end with tube 16. rack 2 with tube 32, and rack 3 with tube 48. The Maxwell front tray will have tubes 1-24. The back tray will have tubes 25-48. A partial rack will impact the last tube number. The tops of the elution tubes will correspond to the numbering on the top of the preprocessing tubes.*

4. Check that the Plunger Tools are on the Tool Rack in Site 2 on the deck.
5. Place Plunger Racks on site 3 and 4 on the deck carrier. If plungers fail to be added to some cartridges during the Maxprep run, they can be manually added prior to Maxwell extraction.
6. Place 1000 µl tip racks in sites 2 and 3 on the tip carrier. These tips are color coded white with a sticker on the deck site.
7. Place 300 µl tip rack in site 4 on the tip carrier. These tips are color coded yellow with a sticker on the deck site.
    - CRITICAL – the correct volume tips must be in the correct location. Service and repair will be required if 300 µl tips are used in the 1000 µl tip sites.
8. Launch the software for the MaxPrep Liquid Handler.
9. ~~Reference the Graphical User Interface (GUI) prompts to ensure the correct placement on the deck of all consumables.~~
10. Select Start > Method > Maxwell FSC DNA IQ Method as named by BCI
11. Follow the ~~Graphical User Interface (~~GUI~~)~~ prompts for *the placement on the*

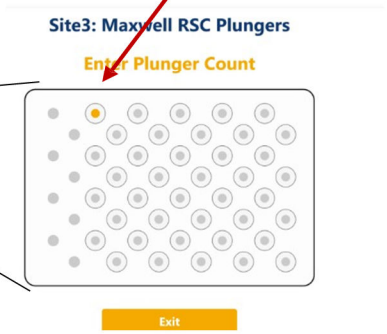
deck of all consumables, samples, reagents, and running the method.
NOTE!!! Ensure **all steps are complete** before Pressing **'NEXT'** to begin sample processing. Do not click backwards or the prior page information ~~will~~ *may* have to be re-entered.



12. Select the appropriate Sample Number by using the slide bar. The Elution Volume (50 µl) is set as a default. When finished, click '**NEXT'**.
13. *Review:*
    a. *Site 1: Heater Shaker – No labware should be Present.*
    b. *Site 2. Tool Rack – Gripper Paddles and Plunger Tools*
    c. *Site 3. Plunger Rack – Enter Plunger Count*
    d. *Site 4. Plunger Rack – Full Plunger Rack*



Site 3: When inserting plunger/ tip count **always select the first available tip** plunger.

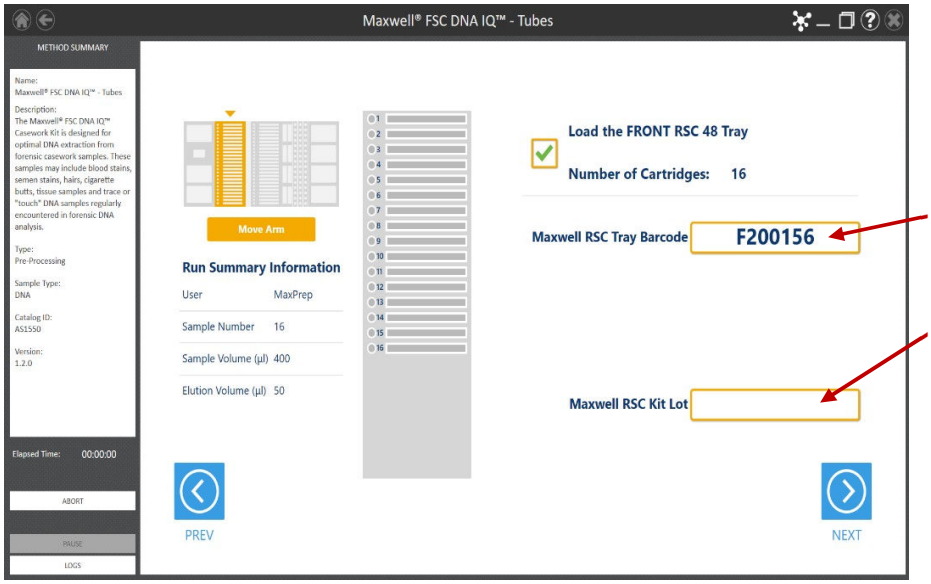


Site 3 and 4: Both boxes need to be **checked to activate the 'Next'** button. Site 4 should always have a

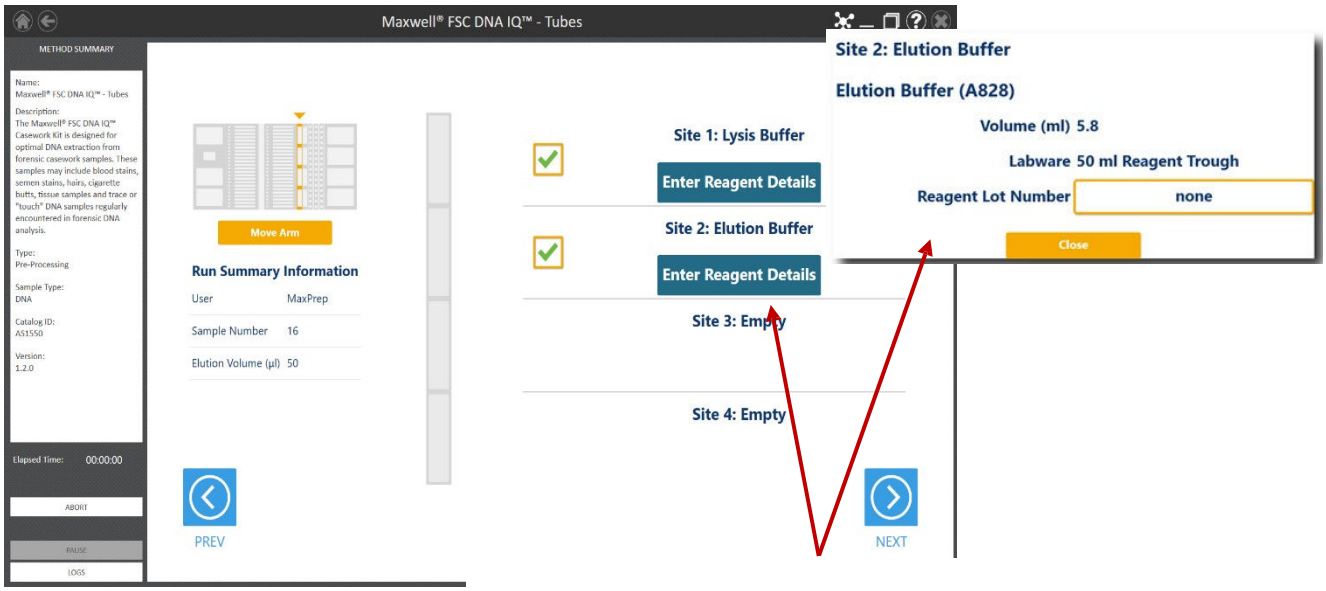
full rack of plungers. When finished, click **'NEXT'**

14. Scan the Maxwell RSC Tray Barcode. This will link the samples scanned in by the Maxprep to the tray for portal entry into the Maxwell instrument.
15. Scan the Maxwell RSC Kit Lot Barcode. This ensures the correct method is run on the Maxwell via the portal.

When finished, click '**NEXT**'



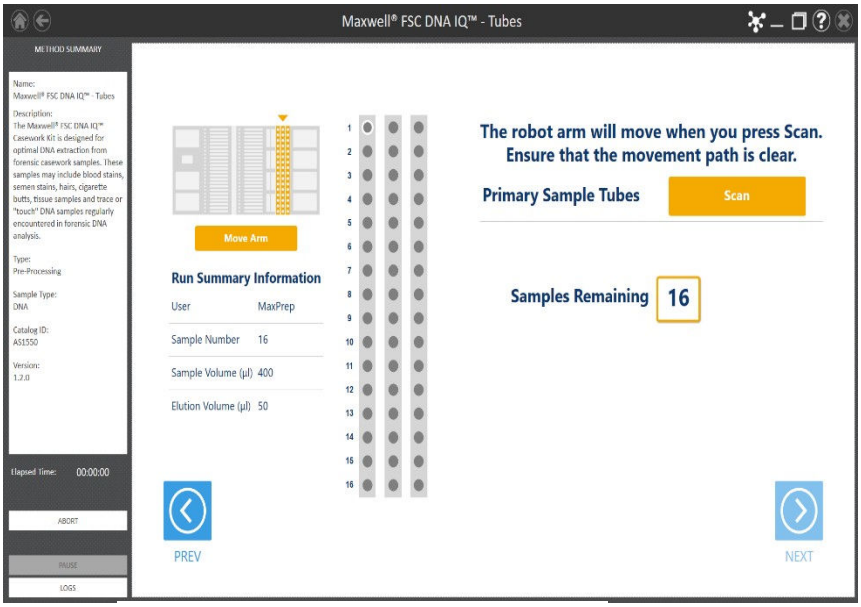
'Tray barcode' will appear first, fill this in with the manual barcode scanner. Then the **'Kit lot'** box will appear again. Use the manual barcode scanner to **scan the QR code on the Maxwell kit box** to complete this section.



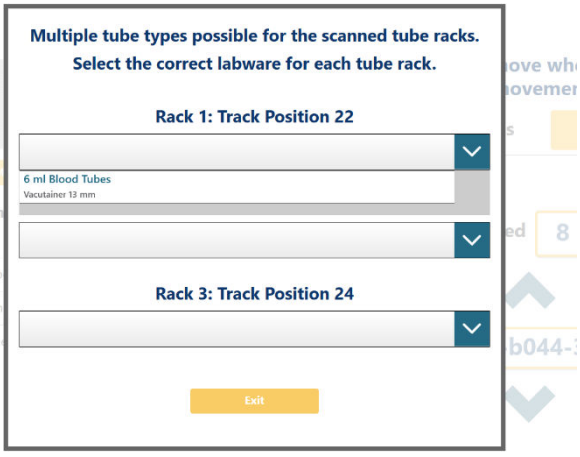
16. Place an appropriate volume of Lysis Buffer (Site 1) and Elution Buffer (Site 2) on the deck carrier. The set-up program will provide the minimum volume of each to include based on the number of samples.

    Note: The lot number for the lysis and elution buffer does not need to be entered. This information can be tracked based on the kit lot number entered previously.
17. Review:
    a. ***Site 1:*** *Lysis Buffer*
    b. ***Site 2:*** *Elution Buffer*
    c. ***Site 3:*** *Empty*
    d. ***Site 4:*** *Empty*

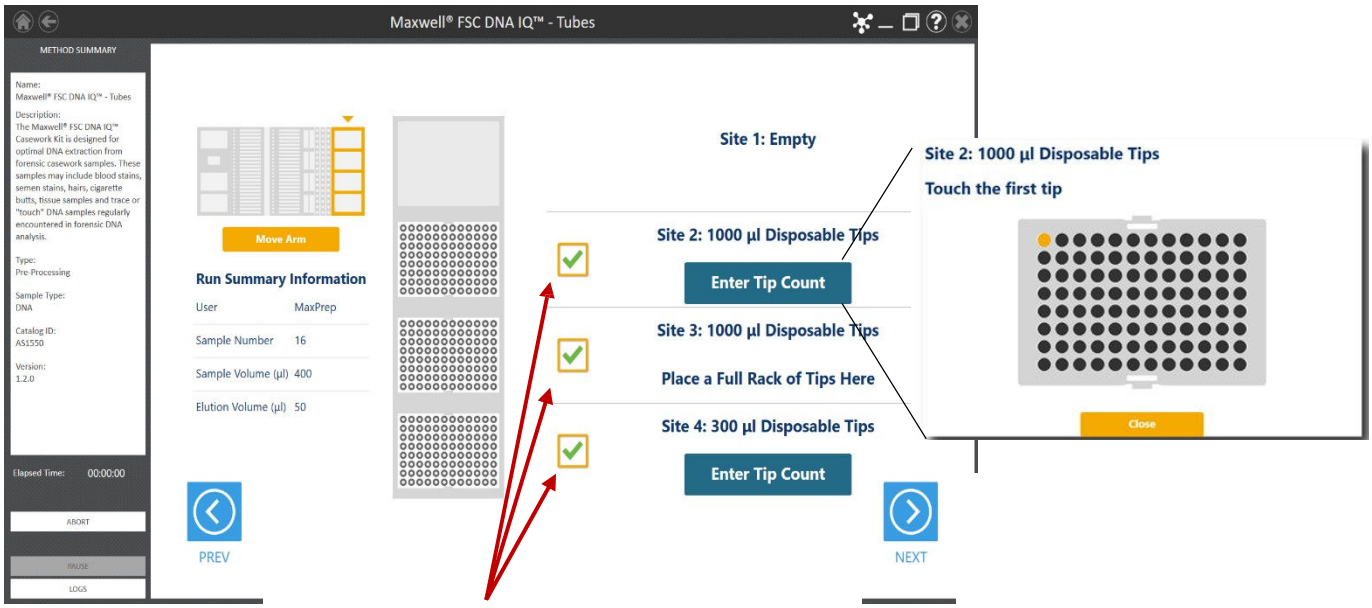
18. If QR codes are used, scan tubes from barcodes or go to 19. Ensure all tubes scan and are in the correct order. If a tube is skipped, it can be manually entered. Make sure the top of the first FB tube matches the top of the first elution tube to make sure they were placed in the tube racks in the correct order. *If QR codes are not used, enter the tube numbers used.*



19. When finished, click '**NEXT'.** Select the tube type (Qiagen Spin and Lyse) for Racks 1-3.



20. Enter the starting information regarding the tips.
    Review:
    a. ***Site 1:*** *Empty*
    b. ***Site 2:*** *1000 µL Disposable Tips (Enter tip count)*
    c. ***Site 3:*** *1000 µL Disposable Tips (full rack of Tips Here)*
    d. ***Site 4:*** *300 µL Disposable Tips (Enter tip count)*



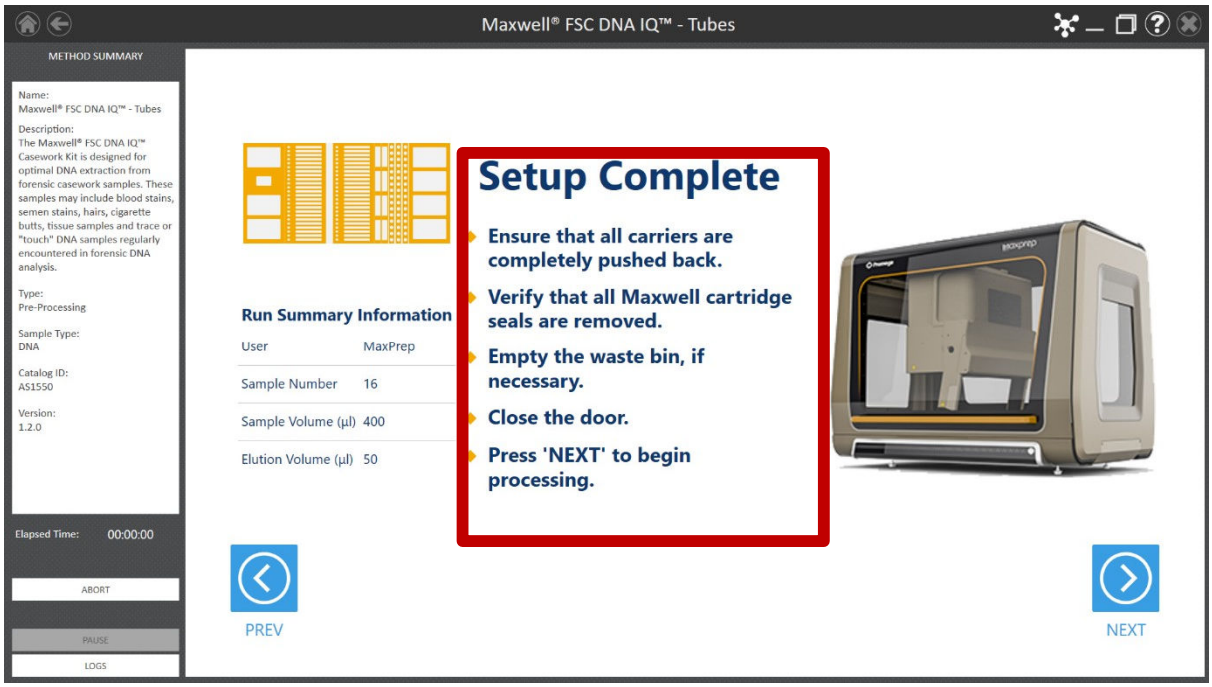
All boxes need to be checked to activate the
'Next' button.

When finished, click '**NEXT'**

21. When the Setup Complete screen is obtained a deck check will be performed by a second person if available, otherwise a self-check will be performed.

Key things to check
-tips in correct spot
-plungers present with full rack on site 4
-reagents in correct order
-tube racks in correct order
-trays seated fully
-tip ejector bar seated fully/properly
-all carrier trays pushed back fully (instrument will not run if not, error message is not clear as to what the problem is)
-this is NOT the time to empty the waste bin. The arm is in the way and there are open samples on the deck

- ~~Tube check - Make sure that the elution tube lid labels in the front tray match the sample tube lid label placed in the first tube rack.~~
- *(1) Check at least one scanned tube QR code against each manifest to ensure that the tubes and racks were loaded correctly. Or (2) At least one tube from each Maxprep tube rack will be compared to the extraction manifests to help ensure that rack contains correct samples according to the manifest.*



22. Click NEXT to start the Maxprep instrument run.
23. Reagents should not be stored on the deck for longer than 1 work day. Empty the waste bin as needed or at the end of the day.

Note: *If using the portal,* the Maxprep designated as the “main” computer should be left on since it is where the portal houses runs.

Maxwell processing (Manual, *Tecan* and Maxprep without Portal)

1. Add plungers into position 8 of the cartridges (closest to elution tube), if not already present.
2. Turn on the Maxwell instrument, then turn on the tablet and open the Maxwell software. The instrument will initialize.
3. Select "Start"
4. Scan the barcode on the Maxwell FSC DNA IQ Casework kit box to open the protocol.
5. Press "Proceed" and then press "OK" to open the door.
6. Load Maxwell tray(s). If using both trays, load the back tray first.
    a. Option 1 – suggested for trays setup with Tecan
        i. Read through the extraction checklist prompt and perform deck check.
        ii. If the checklist items and deck check are correct, press "start" to close the door and begin processing.
        iii. Go to step 7.
    b. Option 2 – suggested for trays either setup manually or with Tecan
        i. Click "cancel" on the extraction checklist prompt in order to manually select the cartridge locations loaded on the tray. The active tray name (front tray or back tray) will be highlighted in blue. Once a cartridge location is selected, it will be highlighted in white.
        ii. Press "Proceed"
        iii. Read through the extraction checklist prompt, which now lists the position numbers of the loaded cartridges and have deck check performed
        iv. If the checklist items and the deck check are correct, press "start" to close the door and begin processing.
        v. Go to step 7.
7. The run will begin automatically
8. When the script completes, select "open door".
9. Cap all elution tubes and place in a 0.5ml tube rack. Add tube labels.

Maxwell processing (MaxPrep with Portal)

1. *Turn on the Maxwell instrument, then turn on the tablet and open the Maxwell software. The instrument will initialize.*
2. *Make sure the portal is turned on.*
3. *Open Maxwell door*
4. *Load Maxwell tray(s). If using both trays, load the back tray first.*
5. *Select "Start"*
6. *Scan the front and back tray barcodes and press "Continue."*
7. *Preprocessing window will pop up showing portal information. Press "Continue."*
8. *DNA IQ casework method will be selected. Press "Proceed."*
9. *Worklist showing cartridges with sample names will appear on screen (there are buttons on the bottom left to look at the front and back tray). Press "Proceed."*

10. Read through the extraction checklist prompt. If all the checklist items are correct press "Start" to close the door and begin processing.
11. The run will begin automatically
12. When the script completes, select "open door."
13. Cap all elution tubes and place in a 0.5ml tube rack. Add tube labels.
14. Final tube check should be completed by another analyst to ensure all tubes are in order and properly labeled according to the manifest before placing in refrigerated temporary storage for TTP.

Maxwell Shut Down

Remove and discard plungers from cartridges prior to removing used cartridges.

1. Remove Maxwell tray from instrument.
2. Discard used cartridges.
3. Return empty tray to Maxwell. The trays may be wiped with ethanol.
4. Press home button in top left corner. Press door close button in top right corner.
5. Press "sanitize" and allow cleaning to run for 1 minute. Green light will go out when script is complete.
6. Log out of software (press X).
7. Turn off instrument.
8. Shut down tablet.

MaxPrep Shut Down

1. Remove and discard reagents
2. Remove pre-extracted sample tubes and check there is no remaining extract. Cap tubes prior to removal to prevent aerosols
3. Press the 'Home' button to return to the main screen and close the software. Wipe deck with Ethanol.
4. If the last user for the day: Select 'Maintenance' and then 'Sanitization' to run the UV script prior to closing software. Turn off instrument. DO NOT turn off computer.
5. Restock tips/plungers if needed
6. Empty waste bin as needed

16.3 DNA Extraction from Miscellaneous Items

While blood and semen are the most commonly known sources of probative DNA profiles, DNA can be obtained from many other useful sources. The following are some of these sources.

16.3.1 Bone (Organic Extraction)

Bones that have been submerged or exposed to harsh environmental conditions for a long period of time may not yield DNA that is amenable for nuclear DNA testing. If teeth are not available, ask for an entire bone, i.e., toes, fingers, ribs or femurs. Request that the medical examiner NOT saw on the bone.

1. Bones previously kept in freezer storage should be brought to room temperature prior to cleaning for sampling. Use a razor blade to remove any tissue and surface layer of the bone. For larger bones like femurs, can soak the bone in 10% bleach for a few minutes. For smaller

bones that are more porous, can clean them with swabs soaked in 10% bleach. Rinse and dry the bone thoroughly (may need to be dried overnight) ensuring no bleach residue remains on bone when dry. This may need to be done in a hood. Avoid sawed ends of the bone.

2. Bleach all work surfaces and tools to be used. Cover all work surfaces, including vice if used, with brown paper or non-static covering. Depending on the condition of the bone, swabbings or scrapings of the marrow may be collected in addition to collection of the samples below:
    a. Femurs- Use a new blade on the hacksaw for each femur. Samples may be taken from the midsection of the bone, but the ends tend to work best. Place a glassine paper underneath the section being sawed in order to collect the powder. Place the powder into extraction tubes, filling each tube about halfway. Depending on bone condition, multiple tubes may be collected.
    b. Toe, finger, and smaller bones- Can sample from the midsection of the smaller bones, but the ends tend to work best. Place the bone on glassine paper and fold the glassine paper over the bone. Use brown paper to wrap around the bone and glassine paper. Using a hammer, separate the ends from the midsection of the bone and return the midsection piece to the evidence packaging. Place the ends on multiple layers of glassine paper, make a paper fold, and pulverize them into powder using the hammer. Collect the powder into extraction tubes, filling each about halfway. Replace the glassine paper during the pulverization process as needed.
3. Go to Step 1 under non-semen extraction for the organic procedure. Consider doubling the amount of Proteinase K and DTT for the master mix. The amount of master mix dispensed into each tube will remain the same as the non-semen extraction protocol states. Additional master mix may be added to cover powder and ensure proper agitation. Consider incubating the samples on the heat block while vortexing every couple of hours before being left to incubate overnight at 56° C.
4. Can combine the aqueous phase from multiple tubes onto one Microcon filter after the phenol step for extraction. Multiple blanks are required to ensure similar treatment as samples. Retain the original tubes containing powder with the extraction batch.

16.3.2 Chewing gum

1. A swabbing of the gum may be used for testing with the organic or Maxwell pathway. Option for organic extraction: (1) Add MasterMix to the piece of gum, heat, remove and retain the gum, proceed with extraction. (2) Place gum in 400 µl SEB (without ProK and DTT) and mash it up for a few moments using a pipette tip or a clean spatula. Warm the tube slightly if the gum is not pliable. Remove the gum from the SEB and retain. Add 10 µl 20 mg/ml Proteinase K and 5 µl 390 mM DTT to the SEB.
2. Continue with either organic or Maxwell extraction.

16.3.3 Cigarette butts

1. Remove ~1/2 of the paper from the filter end of the cigarette butt (avoiding the glue strip). Cut into smaller pieces. A swabbing of the cigarette butt may also be used for testing.
2. Continue with either organic or Maxwell extraction.

16.3.4 Clothing

To obtain the wearer's type: Take a swabbing from an area of the clothing item that would most likely be in contact with the skin of the wearer. Record in the notes what area the swabbing is taken from.

Process as a non-semen body fluid stain. Alternately, use a cutting. **Areas that have been successful are waistbands, sleeve cuffs, coat tags, inner knee areas, inner surface of pant pockets, inside shoes, palm area of gloves, neckbands and hat headbands.** ***Sample collection from heavy deodorant-stained armpit areas may impact Maxwell extraction, consider organic extraction.***

16.3.5 Envelope Flaps

DNA analysts and latent print examiners should consult each other. The envelope should be handled carefully to avoid destroying latent fingerprints. DNA should be removed from the envelope prior to latent print use of humidity chamber, ninhydrin or physical developer.

Option 1 – Cutting from envelope:

1. Cut out a portion of the envelope body in contact with the glue. Individuals vary considerably in the amount of DNA in their saliva, so the total amount of sample required varies. Cut these pieces into smaller pieces, place in an extraction tube. Use more than one tube, if necessary, with consideration of the needed reagent blank controls.
2. Continue with either organic or Maxwell extraction.

Option 2 – Swabbing from envelope:

1. Steam or soak the envelope open.
2. Collect any epithelial cells that may be present by swabbing the glued surfaces. Use SEB to swab the entire glued surface. The cells adhere to the adhesive and it is important to remove as much of the adhesive as possible.
3. Continue with either organic or Maxwell extraction.

16.3.6 Formalin fixed pathology samples (Organic Extraction)

Formalin fixation causes degradation and cross-linking of DNA. The probability of obtaining DNA results is related to the fixative formulation and the amount of time spent in the fixative. In addition, the use of certain cytological stains may also compromise DNA. Formalin–treated products of conception are generally not suitable for DNA testing and should be referred to DNA management. DNA results may be obtained from some pathology specimens. In sampling these tissues, avoid any obviously necrotic tissue. Cut a portion of the specimen from the wax block, trimming away as much wax as possible. Either crush/cut the specimen into smaller pieces and dissolve the remaining wax with two or three changes of xylene substitute followed by two or three changes of alcohol prior to adding SEB. Before adding the ProK and DTT, incubate the sample in SEB at 90°C for one hour with intermittent mixing. Briefly spin then place on ice for 2 minutes. Add DTT and ProK then proceed with the non-semen stain extraction procedure. See detail below.

<u>Paraffin Removal</u>

1. In a fume hood, add 800 µl of xylene substitute to the sample. Allow to incubate for approximately 15 minutes with intermittent shaking. Pellet the sample at full speed for 3 minutes. Remove xylene substitute while avoiding the pellet. Repeat step 1 an additional 1-2 times to remove as much paraffin as possible. Incubation at 56°C may be added to aid in paraffin removal.
2. Add 800 µl of 100% ethanol (v/v) to the sample and vortex. Pellet the sample at full speed for 3 minutes. Remove the ethanol while avoiding the pellet. Repeat step 2 an additional 1-2 times.
3. Air dry the pellet for 5 minutes to evaporate excess ethanol.

De-Crosslink DNA from itself and to proteins by formalin fixation

4. Add 400 µl of SEB to the sample and incubate at 90°C for one hour with intermittent mixing. Briefly spin and then place on ice for 2 minutes.
5. Make a master mix of ProK and DTT then add 15 µl into each sample. Ratio of master mix should be 10 µl 20mg/ml ProK to 5ul 390 mM DTT. Ensure that extra volume of master mix is made to account for pipetting.
6. Incubate the sample at 56°C for 2 hours to overnight then follow the standard phenol extraction procedure.

16.3.7 Hair with roots-free

Organic Extraction

1. If the hair is potentially coated with foreign DNA (i.e. blood from a different person), rinse it in a microfuge tube of Molecular Biology Grade water. Rinse hair briefly in fresh Molecular Biology Grade water.
2. Cut off a 1-2 cm section or less containing the root. Retain the remainder of the hair for possible mtDNA testing.
3. Extract in 400 µl stain extraction buffer with 5 µl 390 mM DTT and 10 µl 20 mg/ml Proteinase K at 56°C for several hours to overnight.

Maxwell Extraction

1. *If the hair is potentially coated with foreign DNA (i.e. blood from a different person), rinse it in a microfuge tube of Molecular Biology Grade water. Rinse hair briefly in fresh Molecular Biology Grade water.*
2. *Cut off a 1-2 cm section or less containing the root. Retain the remainder of the hair for possible mtDNA testing.*
3. *Go to the non-differential Maxwell extraction procedure. Check to ensure that the hairs do not go through the basket after spins. The hair fragment may block the function of pipetting for downstream steps.*

16.3.8 Hair with roots-slide mounted specimens (Organic Extraction)

There are two ways to remove cover slips from items mounted on a slide. Both should be conducted in a fume hood. In each case, the exterior of the slide should first be cleaned with a 10% bleach solution.
Freezing - Place the slide in a small container and pour in liquid nitrogen. Within seconds the cover slip can be removed.
Soak Slide in Xylene Substitute - Soak the slide in xylene for several hours until the cover slip can be slid or pried from the slide. **Caution: this may remove markings from the slide.**
After cover slip removal

1. For hairs, add a drop of xylene substitute to the hair to dissolve the mounting medium. Remove the hair from the slide and soak in xylene substitute for a few minutes to dissolve any remaining mounting medium.
2. For tissue or sperm, allow xylene substitute to flow over the slide to remove the residual mounting media.

3. Rinse the hair or slide briefly in water to remove xylene substitute.
4. For hairs, go to step 15.3.7 under "Hair with roots-free".
5. For tissue or sperm, swab the stain from the slide.

16.3.9 Soft tissue

Follow the non-semen method to extract DNA from fresh tissue. Generally, a piece 1-3 cubic mm equivalent is sufficient; use less for products of conception. *Prior to collecting sample for products of conception rinse the cutting with sterile water before placing into tube. Add 1000ul TE to cutting, vortex and spin down at maximum speed for three minutes; repeat up to two more times. After final wash remove TE and continue with either organic or Maxwell extraction. For more developed products of conception, consider sampling a digit.* If the Microcon filter becomes plugged, it is most likely due to excess DNA.

16.3.10 Teeth (Organic Extraction)

1. When possible, use a tooth with an intact root. Depending on the state of the tooth, may soak in 10% bleach solution for a few minutes or use swabs soaked in 10% bleach to clean the tooth. May need to remove tissue/decay. Rinse and dry thoroughly.
2. Bleach hammer and work surfaces. Place the tooth on multiple layers of glassine paper and create a paper fold. May be necessary to secure edges of the paper to work surface.
3. Hit the tooth with a hammer to separate the root from the enamel portion of the tooth (be careful to contain the tooth pieces when hitting with a hammer). Return the enamel portion to the original evidence packaging.
4. Place the root portion on multiple layers of glassine paper and create a paper fold. Pulverize the root with the hammer.
5. After hitting the root multiple times, separate the powder from the larger pieces of tooth. Collect the powder in extraction tubes. Replace the glassine paper during pulverization as the tooth becomes embedded in or breaks through the paper.
6. Repeat step 5 until the root has been pulverized into powder or until enough powder has been collected to fill an extraction tube to the halfway mark, if possible
7. Go to Step 1 under non-semen extraction. Add additional master mix as needed to cover powder in tube and ensure proper agitation.
8. Can combine the aqueous phase from multiple tubes onto one Microcon filter after the phenol step for extraction. Retain the original tubes containing powder with the extraction batch.

16.3.11 Toothbrushes

1. Swab the entire head and bristle area of the brush with swabs moistened with water or SEB. Alternatively, cut a few bristles off of the toothbrush and insert into a tube.
2. Continue with either organic or Maxwell extraction.

16.3.12 Alternate extraction method for cartridge cases

1. If possible for cartridge cases, wedge a small rubber stopper or wooden dowel in the cartridge cases to use as a handle.
2. Moisten a cotton swab with a drop of 500 mM EDTA. Using only the tip of the swab, scrub all of the outer surfaces of the cartridge cases, being careful to get into the indentations on and around the base. Repeat with a dry cotton swab.
3. Cut the two swab tips into 250 µl low copy number (LCN) buffer.

4. Add 10 µl of 20 mg/ml Proteinase K and incubate 56^{o}C 2 hours.
5. Incubate 99^{o}C 10 minutes.
6. Add 100 µl of 20 ng/ µl salmon sperm DNA to the filter of a Microcon.
7. Add the extraction solution.
8. Spin until dry/nearly dry. Start at ~4500 RPM.
9. Wash once with 100 µl TE.
10. Recover in 50 µl TE.

17 DNA Quantification Using Real Time PCR with the Quantifiler Trio Kit

Be familiar with Quantifiler Trio Kit literature before proceeding.

17.1 Quantifiler Assay Background

Quantifiler Trio DNA quantification kit (Life Technologies) is designed to quantify the total amount of amplifiable human (and higher primate) DNA and human male DNA in a sample. The results from using the kit can aid in determining: 1) If sufficient human DNA or human male DNA is present to proceed with short tandem repeat (STR) analysis, 2) Degree of DNA degradation, and 3) How much sample to use in STR analysis applications.

The DNA quantification assay combines four 5' nuclease assays. Each amplification well contains small human DNA, large human DNA, and human male DNA target-specific assays and an internal PCR control (IPC) assay. The target-specific assays consist of primers for amplifying the small human DNA (80 bases), large human DNA (214 bases), or human male DNA (75 bases) and one TaqMan (MGB or QSY quencher) probe labeled with VIC dye (small human DNA), ABY dye (large human DNA) or FAM dye (human male DNA) for detecting the amplified sequence. The IPC assay consists of IPC template DNA (a 130 base synthetic sequence not found in nature), primers for amplifying the IPC template DNA, and one TaqMan QSY quencher probe labeled with JUN dye for detecting the amplified IPC DNA. Successful amplification of the PCR control and the target DNA indicates that amplifiable DNA was detected. A scenario where the PCR control amplifies, but the sample does not, could indicate that sufficient amplifiable DNA is not present. In the absence of robust target DNA amplification, this assay can predict inhibition if the IPC also does not amplify or has a high C_T.

17.2 Preparation of Standard Curve Samples

1. After allowing for placement of the five recommended standards and the dilution buffer (blank) in duplicate, *up to* 84 samples fit on the plate.
2. Start the computer and turn on the Life Technologies 7500 instrument.
3. Required Material
    a. Low-bind microfuge tubes
    b. Trio DNA Standard
    c. Trio DNA Dilution Buffer
4. Refer to the chart for volumes.
5. Dispense the dilution buffer into the labeled tubes.
6. Vortex the DNA stock 3-5 seconds and quick spin before opening.
7. Prepare Standard 1, vortex and quick spin.
8. Using a new pipette tip, prepare Standard 2, vortex and quick spin.
9. Repeat the previous step until all standards are prepared.
10. Store prepared standards up to 1 week at 2-8°C in tubes.

Standard	ng/µl	Volumes of 100 ng/µl stock and dilution buffer (may be scaled for a specific volume)	Dilution
1	50	10 µl stock + 10 µl dilution buffer	2x
2	5	10 µl std 1 + 90 µl dilution buffer	10x
3	0.5	10 µl std 2 + 90 µl dilution buffer	10x
4	0.05	10 µl std 3 + 90 µl dilution buffer	10x
5	0.005	10 µl std 4 + 90 µl dilution buffer	10x

17.3 Prepare Trio Master Mix and Set up the Plate

1. Required Materials:

    a. Trio Primer Mix
    b. Trio Reaction Mix
    c. Disposable tray or tube for preparing master mix
    d. 96-well optical reaction plate
    e. Extracted DNA samples
    f. DNA quantification standards dilution series
    g. Trio Dilution Buffer
    h. Optical adhesive cover

    Optional: Quantifiler Automation Enhancer (ABI A31287)
2. Thaw the Trio Primer Mix, vortex 3-5 seconds, spin briefly.
3. Gently vortex the PCR Reaction Mix, spin briefly. Optional, add 1 µl of Quantifiler Automation Enhancer to the PCR Reaction mix when first opened. The enhancer helps minimize bubbles in automated set-up.
4. Use the chart to calculate the volume of the components needed to prepare the reactions.

Trio Master Mix Calculator		
Primer Mix	Number of samples + controls + 5	X 8 µl
Reaction Mix	Number of samples + controls + 5	X 10 µl

5. Mix components thoroughly and dispense 18 µl of the Quantifiler Trio PCR mix into each reaction well to be used on the QF plate.
6. Add 2 µl of each standard, in duplicate, to columns 11 and 12. Then, add 2 µl of samples and negative control (DNA Dilution Buffer) to the control wells.
7. Seal the reaction plate with the optical adhesive cover using a plate comb and ensuring the seal is secure all the way around the plate. Remove the tabs on the seal.
8. Centrifuge the plate at 3000 rpm for about 20 seconds. If a centrifuge is not available, gently tap the plate seated in a base on the table to remove bubbles from the wells.

17.4 Procedure if no template has been created

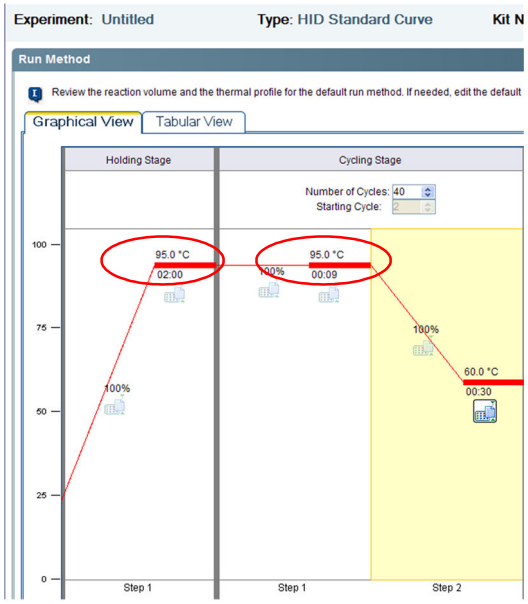
1. Open the HID Real Time PCR Analysis software.
2. Log into the software as “Guest”.
3. In the Home screen, click the “Quantifiler Trio” icon.
4. In the Experiment Properties screen, enter a name for the experiment.
5. In the left navigational panel, click Setup > Plate Setup.
6. To define the samples, click Add New Sample in the Define Samples area on the right side, then type the name for the sample. Select the sample type (Unknown is the default for new samples). Save the sample and then repeat for each sample.
7. Click the Assign Targets and Samples tab. Targets are automatically assigned and the standard quantities are automatically specified.
8. Assign the samples to the plate wells.
9. Select wells.
10. In the Assign sample(s) to wells section to the left of the plate layout, locate the desired sample and select the checkbox in the Assign column next to the sample name. The target for each sample is set by default.
11. Repeat for all samples.
12. In the left navigational panel, click Setup > Run Method to view the parameters. The parameters are automatically specified.
13. Save the file in the experiments folder as an eds file.

17.5 Setting up a plate document on the 7500 Real-Time PCR instrument

1. If the HID Real-Time Analysis Software is not already started, select the HID Real-Time PCR Analysis Software located on the computer desktop and turn on the 7500 instrument.
2. Log into the software as “Guest” and then click “Quantifiler Trio” icon.
3. Select File > Import, then select Browse to navigate to the appropriate Quantifiler Trio text file, then click Select. Click Start Import, then click Yes, and finally click Okay.
4. When all wells have been checked for the correct detectors and sample names, save the file in the format of “MMDDY_Batchtype_Initials”.

17.6 Preparing the Thermal Cycler

1. In the Experiment Menu, select Setup > Run Method.
2. Verify the sample volume is 20 µl and the thermal profile is the following:



17.7 Running the Reactions

1. The computer and instrument are on and the software is open.
2. Press the tray door to open it.
3. Load the plate into the plate holder aligning so that well A1 is in the upper-left corner and the notched corner of the plate is in the upper-right corner.
4. Apply pressure to the right side of the tray at an angle to close the tray door.
5. Create an experiment for the run or use a template.
6. Click Start Run and it will prompt to save the experiment.
7. Before leaving the instrument unattended, verify that the run has begun by checking that the run time clock has begun to count down. During the run, you will see the amplification plot built.

17.8 Checking and Analyzing the Run

1. Open the run file and select Analysis Settings in the upper-right corner. Click the CT Settings tab.
2. Verify the threshold is 0.1 and the baseline is set for automatic, then click Apply Analysis Settings.
3. Click Analyze.
4. In the left navigational panel, click Analysis > Standard Curve.
5. In the Target drop-down list, select All.
6. Verify that slopes are between -3.0 and -3.6 (optimal = -3.3) and that the R2 value is >.98. Delete points from the curve to achieve the proper slope. **Reanalyze if points are removed.** If

values cannot be brought within range, consult with an FSC or DNA management. The results may still be useful but some troubleshooting is necessary.

7. The gap between the Small Autosomal, Large Autosomal and Male CT values may vary depending on the relative slopes of the targets and instrument performances.
8. Small amounts of background in the negative control or blanks do not invalidate results.
9. After the run has been analyzed and inspected for quality, save the file.
10. When printing results for Trio performed manually, do not print results for the standards or dilution buffer wells. These wells are checked on the .pdf version during analysis and review but are not printed for the case file.
11. If no name is entered in the Experiment Name field of the Experiment Properties screen, the experiment name on the report is "Untitled."

17.9 Exporting data

1. In the Experiment Menu, select Analysis. Click any Analysis screen, then click either View Plate Layout or View Well Table. Highlight the wells to export.
2. In the toolbar, click Export.
3. Under the Export Properties table, select Results as data to export, and then select it to be exported as One File. Select .xls as the File Type and specify an export location.
4. Click Start Export. When export is complete, close the Export tool.

17.10 Assessing quality of results

1. The closer the R2, slope and y-intercept values are to ideal, the more informative the quantitation results. If these values are off, consult with an FSC or DNA management. The results may still be useful but some troubleshooting is necessary.
2. The R2 value describes how well the data points of the standards fit the curve. An R2 = 1 is a perfect fit. For best results, the R2 should be no less than 0.98. If it is less, outlier data points will be seen. Remove any data points that are far off the curve.
3. The slope describes how well the reaction is working. The theoretical maximum slope of -3.3 corresponds to a doubling of DNA at each cycle. In practice, the ideal slope should be -3.0 to -3.6. A more negative slope (i.e. -3.7) may reflect a poorly performing reaction or bad data points in the curve. Once bad data points have been removed, make no further adjustments on the slope. Outlier data points to the right of the curve make the slope more negative. Those to the left of the curve make it more positive. Values in the middle of the range (those samples quanting between 0.5 and 2.0) will be least affected by variant slopes.
4. The y-intercept value is the cycle number when the 1.0 ng point of the standard curve crosses the threshold, generally falls in the range of 25.5-28.5 for small autosomal, 23.5-26.5 for large autosomal, and 25-28 for male. Small variation in the y-intercept may occur with different lots of standard curve DNA. An outlier Y-intercept may signal a problem with the standard curve that should be taken into consideration during interpretation.

5. A low Y-intercept may result in a falsely low quant and subsequent overloading of sample on e-gram. The cause may be a difference in a new batch of standard curve DNA or in measuring error in the first step of the standard curve dilution.
6. When the Y-intercept is different from the value normally obtained, consider increasing or decreasing the amount of DNA input into the Globalfiler amplification.
7. The CT for the IPC generally falls in the in the range of 27-29 cycles.
8. Where the sample DNA input is very high, the IPC CT will be slightly higher, reflecting competition for amplification reaction components.
9. A reaction where the IPC and the other targets are undetected has failed to amplify, most likely due to a soluble inhibitor in the casework DNA sample. Consider performing an additional amplification of a dilution of these samples.
10. A reaction where the IPC is very low or undetected and the sample seems to have amplified adequately is partially inhibited. It may be helpful to perform a second amplification of a sample dilution. The manipulation blank should also be diluted and amplified.
11. A reaction where the IPC is within normal range and the sample is very low or undetected is an indication of the absence of any amplifiable human DNA.
12. When y-intercept, slope or line fit fall outside of ideal ranges, DNA quantity data is generally still sufficiently accurate to produce good typing results. Only in the most extreme circumstances would the benefits of repeating the Quantifiler test outweigh the additional time and sample resources required. This decision should be made in consultation with DNA management.

17.11 Assessing quantity of DNA present in samples

1. After determining the quality of the results, determine whether sufficient DNA is present to proceed. The quantification value from the small autosomal target should be used to determine STR input.
2. The Trio Kit can detect DNA concentrations under 5 pg/µl. Significant variability in results below this level are due to stochastic effects.
3. The Degradation Index is a ratio of small/large target. As the value increases, a higher amount of degradation can be inferred. Degradation ratios of 3 or above may prompt increasing the Globalfiler amplification target value. The Degradation Index can be affected by the degree of degradation of the large autosomal target DNA and the presence of PCR inhibitors.
4. The IPC CT flag is triggered for “undetermined” or very high values and may indicate inhibition.
5. The Quality Index is the concurrent consideration of both the degradation index and the IPCCT flag. This may influence downstream sample clean-up or targeting decisions.

Potential Interpretations of Degradation and Quality Indexes		
IPCCT flag?	Degradation Index	Interpretation
No	<1	DNA not degraded or inhibited
	1-10	DNA slightly to moderately degraded
	>10 or blank	DNA is significantly degraded
Yes	>1 or blank	DNA is degraded and/or inhibited

18 Globalfiler STR Profiling (see previous manuals for Identifiler STR kit interpretation guidelines)

18.1 Introduction

STR loci are amplified using the polymerase chain reaction (PCR). The PCR products are then separated by capillary electrophoresis according to their size. The Globalfiler PCR Amplification Kit amplifies 24 loci in one PCR reaction, including 21 autosomal loci (D3S1358, vWA, D16S539, CSF1P0, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, and D2S1338), one Y-STR (DYS391), one insertion/deletion polymorphic marker on the Y chromosome (Y Indel), and Amelogenin.

During capillary electrophoresis, the use of multicolor dye-labeled primers allows multiple loci, including those with overlapping fragment sizes, to be analyzed in a single injection. As the PCR products travel through the capillary they are separated according to size. As the DNA fragments electrophorese past a detection window, a laser excites the fluorescent dyes. The light emitted from the excited dyes is split and then reflected onto a CCD (charge coupled device) camera. The CCD camera then translates the intensity into real-time data which is observed as peaks on a computer screen. An internal size standard is loaded with each sample to allow for automatic sizing of the PCR products.

18.2 Amplification of Globalfiler

The amplification kit for Globalfiler contains all the necessary reagents.
Protect the primers, amplified DNA, allelic ladder, and size standard from light when not in use.
After first use, store the reagents at 2-8°C.

Samples and blanks that have evaporated may be reconstituted to the expected remaining volume. The same aliquot TE used for reconstitution should be used for the negative amplification control or start a TE control blank. Log the volumes added in the case documentation.

1. The target DNA quantity is 1.0 ng and the sample volume is 15 µl. For samples below or equal to 0.067 ng/µl, use 15 µl. More concentrated DNA may require dilution.
2. TE 10:0.01 may be used as the diluent.
3. Consider targeting more DNA where the degradation index from Quant Trio is greater than or equal to 3.
4. Run positive (diluted 1 part positive:2 parts TE – this ratio can be adjusted based on apparent concentration of the kit positive control) and negative controls (TE) with each set of samples amplified. Positive control samples include current or previous lot kit controls or BCI NIST-traceable DNA.
5. Label the controls as follows:
   Pos-amp date-initials Example: Pos_100224_JMC
   Neg-amp date-initials Example: Neg_100224_JMC
6. Thaw the Master Mix and Primer Set, vortex and spin briefly before opening.
7. Prepare the reaction mixture:
   Number of samples X 7.5 µl of Globalfiler Master Mix
   Number of samples X 2.5 µl of Globalfiler Primer Set
8. Mix the components thoroughly in a micro centrifuge tube and spin briefly. Dispense 10 µl reaction mixture into each well of a MicroAmp Optical 96-Well Reaction Plate.

9. Pipette 15 µl of sample DNA into the wells.
10. Seal plates with a clear thermal seal using a plate comb and ensuring the seal is secure all the way around the plate. Leave the tabs on the seal.
11. Centrifuge the plate at 3000 rpm for 20 seconds.
12. Place the PCR tubes or plate in the thermal cycler and start the program. Amplify with the following parameters for 29 cycles:

Parameter	Temp °C/time
Hold	95/1 min
Denature	94/10 sec
Anneal/Extend	59/1:30 min
Final Extension	60/10 min
Hold	4/∞

13. After amplification, store protected from light. Refrigerate up to two weeks; freeze for longer periods.

18.3 Preparation of the 3500xL for Electrophoresis

Maintenance Wizards perform maintenance functions:

1. Install a capillary array
2. Remove bubbles
3. Wash pump chamber and channels
4. Fill array with fresh polymer
5. Replenish the polymer
6. Change capillary array
7. Shutdown the instrument

3500 components for Globalfiler Kit	
Dye set	J6
Matrix set	DS-36
Capillary	36 cm
Polymer	POP-4
Formamide	Hi-Di
ILS	LIZ 600

18.4 Starting the Software

1. Turn on the 3500xl by pressing the power button on the front of the instrument. Wait for the green light to appear.
   Turn on the computer and log onto the computer and then log into the 3500 software (the icon looks like the instrument). The order of start-up is "ICS"-Instrument, Computer, then Software. The order of shutdown is the exact opposite, Software, Computer, Instrument.
2. Launch the application via **Start > Programs > Applied Biosystems > 3500.**
3. The Dashboard is the first screen displayed. It shows gauges, instrument and consumable information, and notifications.

18.5 Re-Starting the Computer

1. Turn on the computer and wait for the login screen to show.
2. Turn on the 3500xl by pressing the power button on the front of the instrument. Wait for the green light to appear.
3. Log onto the computer and then log into the 3500 software (the icon looks like the instrument). or
   Launch the application via **Start > Programs > Applied Biosystems > 3500.**

The Dashboard is the first screen displayed. It shows gauges, instrument and consumable information, and notifications.

18.6 Checking maintenance notifications

1. Review the Maintenance Notification pane and perform any scheduled tasks. Click the checkmark to mark any completed tasks as performed.
2. Perform any routine maintenance tasks not listed in the Maintenance Notification pane.

   "Days remaining for buffers" updates only when **Refresh** is clicked or when a run is started.

3. Click **Refresh** to update consumable status.
4. Check the consumables gauges.
5. Check the fill levels on buffers. Verify that the buffer level is at the top of the fill line and the septa are intact.
6. As needed, replenish polymer, change buffers, and change the capillary array.

18.7 Preparing the 3500xl

1. In the Dashboard, check consumables and buffers before each run.
2. Press the tray button to access the cathode buffer container. The cathode and anode buffer containers need to be changed after 14 days on the instrument. When changing the anode buffer, make sure the entire buffer is in the larger reservoir.
3. Check the polymer.
   - If it is less than two weeks old and sufficient to finish the runs (25-40 µl per injection), check for bubbles in the blocks and tubing.
   - If it is less than two weeks old but not enough to finish the runs OR is more than two weeks old, use the Replenish Polymer wizard.
4. If a capillary array has been installed or replaced, perform a spatial and spectral calibration (see User Manual).
5. If a new dye set is being used, the laser or CCD camera has been realigned, or if there is an increase in spectral separation (pull-up/pull-down peaks), perform a spectral calibration (see User Manual).
6. Set the oven temperature to 60°C and click **Start Pre-heat.** Pre-heating helps minimize first-run migration rate effects.
7. Check the pump assembly for bubbles. Remove bubbles using the wizard as needed.

18.8 Loading Samples/Allelic Ladder

1. An injection is a defined set of 24 wells on a 96-well reaction plate. The injections are ordered vertically on the plate, so that wells A1-H3 are injected first, A4-H6 are injected second, etc.
2. Prepare the formamide: size standard mixture
   a. Use 0.4 µl GeneScan 600 LIZ and 9.6 µl Hi-Di formamide per sample.
   b. Vortex and spin briefly.
3. Add 10 µl of formamide: size standard mixture to the wells in blocks of 24 so that all capillaries are submerged during the run.

4. Add 1 µl of sample or allelic ladder (1 ladder per injection) to the wells and cover the plate with septa.
5. Centrifuge the plate to ensure the well contents are mixed and collected at the bottom.

18.9 Preparing the Plate Assembly

1. Place the 96-well plate into the plate base.
2. Snap the plate retainer onto the reaction plate and plate base.
3. Verify that the holes of the plate retainer and the septa are aligned.
4. Place the plate assembly onto the autosampler with the labels facing the instrument door and the notched corner of the plate in the notched corner of the autosampler.
5. Close the instrument door.

18.10 Check instrument status and Create a plate

1. Check instrument status in the Dashboard. Temperatures will be shown in green once they reach the set point.
    a. Preheat oven 30 minutes if the instrument is cold.
    b. On the Dashboard, click **Create New Plate (or Create New Plate From Template** to display the Open Plate Template from Library dialog box.)
2. Enter the plate name (i.e. mmddyy.gf/iq/manual.initials). Or if Select the template labeled " ", then click **Open**.
3. In the Define Plate Properties screen, select HID, 96 wells, 36 cm for capillary length, and POP4 for polymer.
4. Save plate by clicking the "Save plate" button and then "save".
5. Press the "Assign Plate Contents" button at the bottom of the screen.
6. To add samples manually, double click on a well under Table View and not under Plate View to type in the sample name to ensure samples are assigned to correct wells.
7. To import a txt. File, click on "Import" to import the CE Set-up file. Browse to the correct file and import. Delete/edit any wells that are not needed by right clicking on the well and then click "delete".
8. Under Assays, click "Add from Library", select "GF_POP4_24s_xl" and then add to plate or close box.
9. Under File Name Conventions, click "Add from Library", select "3500xl_samplename", then add to plate or close box.
10. Under Results Groups, click "Add from Library", select "Globalfiler Local", add to plate or close box.
11. Double click on a well and click on the assays, file name conventions, and results group name to ensure that all wells contain a blue circle.
12. Click **Link for plate run** at the bottom.

18.11 Linking the plate

1. Access the Load Plates for Run screen (from the Assign Plates for Run screen, click **Link Plate for Run**; from the Dashboard by clicking the **Main workflow arrow** and then selecting **Load Plates for Run,** or from the navigation pane by selecting **Load Plates for Run).**
2. Review the consumables information and the calibration information.

3. Enter a Run Name (mmddyy.gf/iq/manual.initials).
4. Link the plate. By default, the plate in position A is selected. As needed, click **Switch Plates** to assign the plate to the other position in the autosampler.
5. Click either **Create Injection List** or **Start Run**.

18.12 Loading the plate and creating an injection list

1. Access the Load Plates for Run screen (via the **Assign Plate Contents** screen by clicking **Link Plate for Run**; the navigation pane by selecting **Load Plates for Run** in the navigation pane; or the Dashboard by clicking the **Main workflow arrow**, then selecting **Load Plates for Run** in the navigation pane.). Review the consumables and calibration information and ensure the status is ready for a run.
2. Enter a Run Name.
3. Clicking "Load Plates for Run" on the Assign Plate Contents screen automatically links the plate.
4. If needed, click **Unlink**, then follow steps to link the plate.
5. Do either of the following: Click **Create Injection List** or click **Start Run.**

18.13 Review and modify the injection list

1. The injection list can be modified from the Preview Run screen before the run is started.
2. In the Preview Run screen, click the icon above the plate to specify the attributes to display in plate view.
3. Click the plate tabs to display Plate A or Plate B.
4. To make modifications before or during a run, select an injection, then click **Move Up, Move Down,** or **Delete** as needed.

18.14 Running the plate

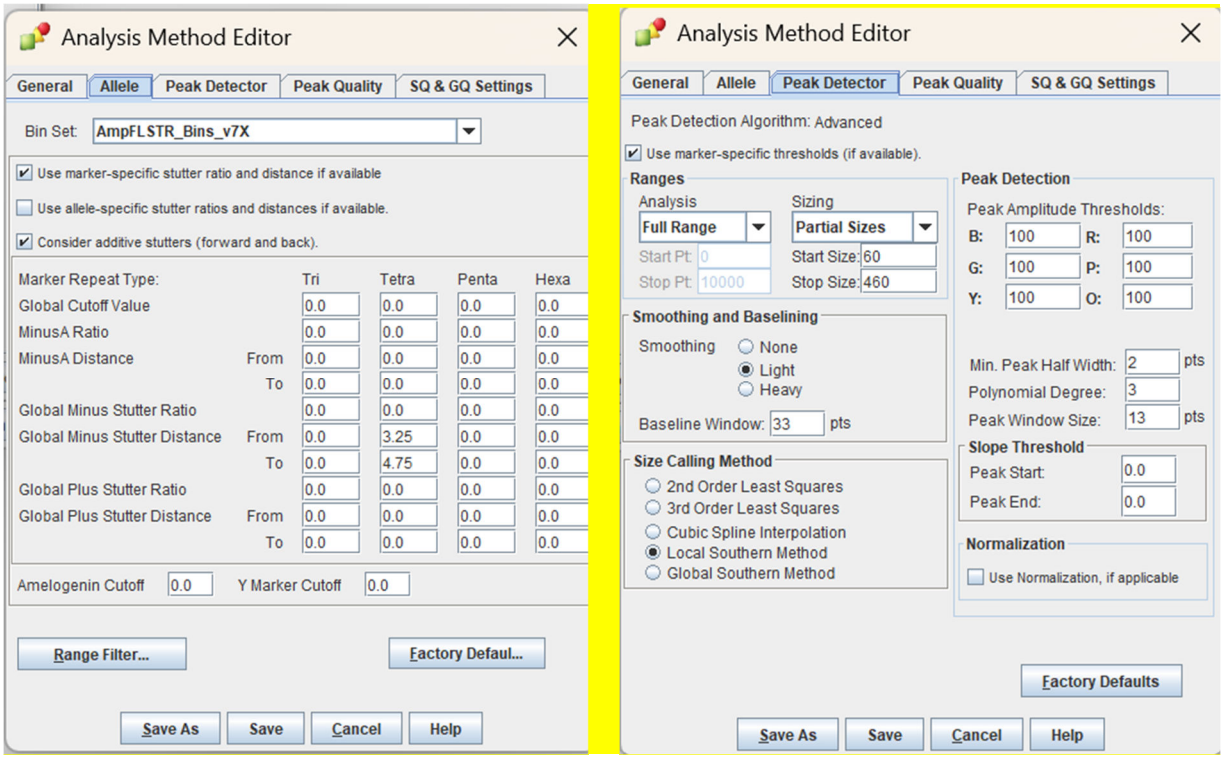
1. A run can be started in either the Load Plates for Run screen or the Preview Run screen.
2. When the injection list is configured, click **Start Run.**
   Note: When the instrument door is closed, it takes approximately 10 seconds for the instrument to initialize. Do not start a run until the instrument status light is green.
3. Save Files to the network.

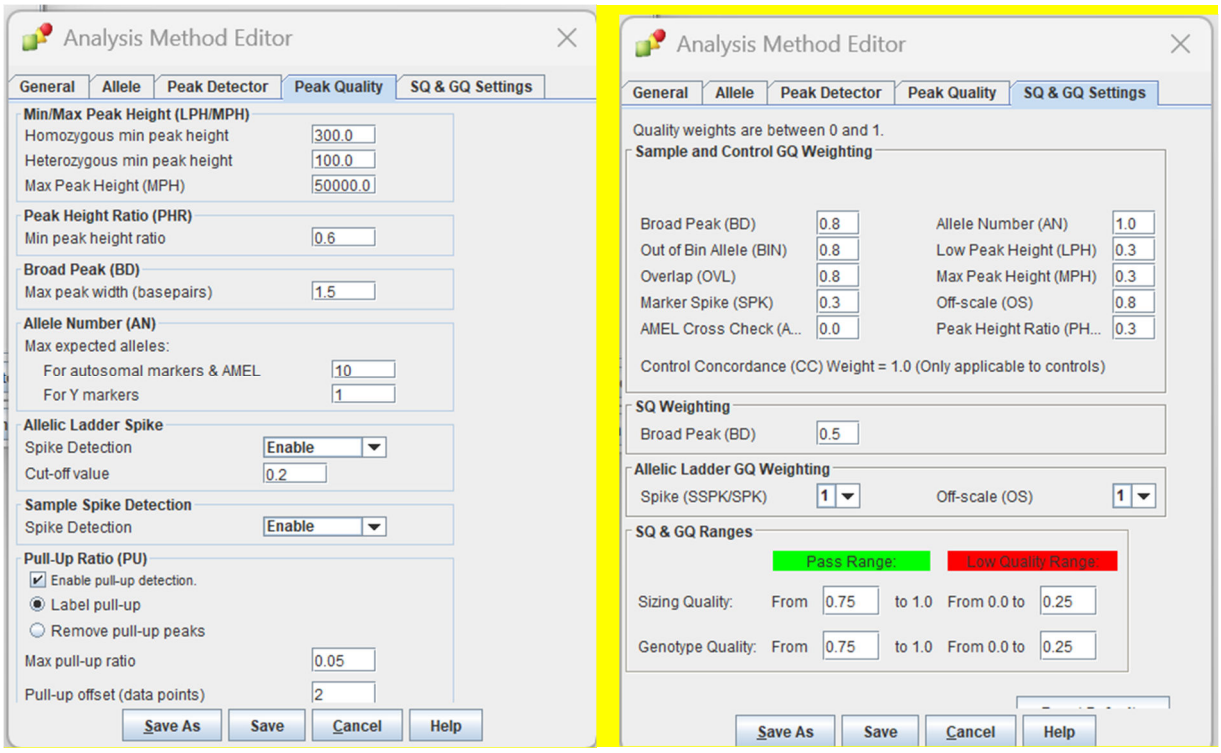
18.15 Analyzing Globalfiler with Genemapper IDX v1.7 software

18.15.1

1. *Follow the naming convention procedure for projects of (mmddyy.gf/iq or std/manual.initials/3500#.run date).*

2. *Globalfiler Analysis settings:*
   a. *Evidence*
      Analysis method: Globalfiler 100 RFU
      Panel: Globalfiler_Panel_v1.2X
      Size Standard: GS600_LIZ_(60-460)
      Bin Set: AmpFLSTR_Bins_v7X
      Additive stutter for peaks in forward and back positions will be selected
      See additional settings below:

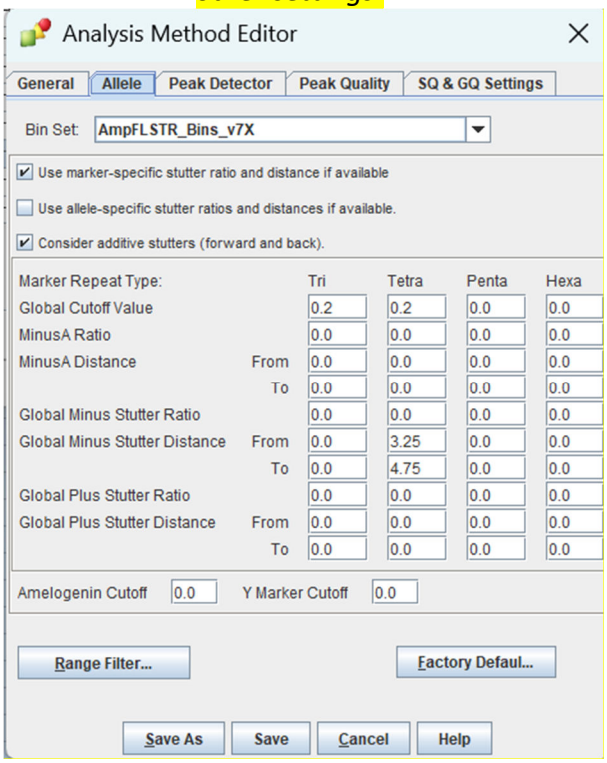




b. Reference Standards

Analysis method: Standards GF 20% 100 RFU

Panel: Globalfiler_Panel_v1.2X
Size Standard: GS600_LIZ_(60-460)
Bin Set: AmpFLSTR_Bins_v7X
See additional settings below for the "Allele" tab. Use the settings in 2 above for the other settings.



3. *Turn on the marker-specific stutter filters for N-2, N-3, N-4, N+3 and N+4.*
4. *The analysis range can be changed from "full range" to a more "partial range" to avoid the primer front and/or if sizing issues arise with the GS600 LIZ. The first allele bin is located after the 60 bp peak and is located at D2S441. Sizing needs to include the 60 bp peak as well as all of the other LIZ peaks through 460 bp to ensure correct sizing results.*

18.15.2 *Printing Electropherograms*

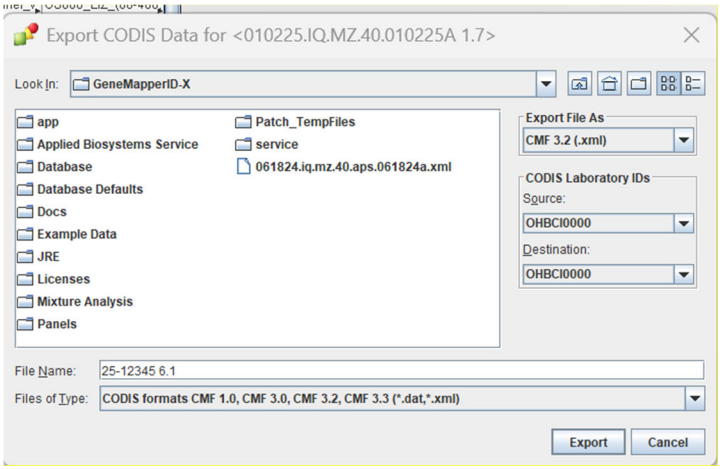
1. *The sample plot options selected for header labels on electropherograms will include the following:*
   a. *Analysis Method*
   b. *Panel*
   c. *Size Standard*
   d. *Analytical Threshold*
2. *These options will be checked to ensure the appropriate Analysis Method, Panel, Size Standard, and Analytical Threshold were used during technical review.*

18.15.3 *Directions for Export from Genemapper v1.7 for CODIS Upload of Single Source Profiles*

1. *Once the Genemapper project has been edited, reviewed and saved for casework, make an export file of the samples. At the top table setting drop down select "CODIS export"*

Sample Type	***GMIDX – Specimen Category***
Suspect	*Unreviewed Suspect*
Forensic Unknown	*Unreviewed Forensic*

2. *Set Specimen category in Genemapper to the correct GMIDX category **only for your samples**. Must be single source profile and have only those alleles for CODIS entry present (no stutter/artifact alleles called).*
3. *Save the project. Go to 'File' and select 'Export Table for CODIS…' to create a .CMF 3.2 (.xml) file.*
4. *Replace all ORI OHBCI0000 with OHBCI0001 for the London casework lab and OHBCI0028 for the Richfield lab– this ORI should match the lab where the export file is created; need to add both ORIs to the dropdowns if not already there and remove SDIS ORI.*
5. *Change the File Name to save as the Case #/Specimen #.*



6. *The export file will insert the GMIDx 1.7 user that is logged in when generating the file. If the analyst creates a new GMID username it **must be** the same as their CODIS user ID or it will need to be edited prior to import.*
7. *Save this temporary file to a known location.*
8. *Log into CODIS.*
9. *Open the analysts' workbench.*
10. *Open Specimen Manager.*
11. *Click on the  "Import" icon. Browse to the thumb drive and select your .CMF file.*
12. *Click on the "Import STR Files" category in Message Center.*
13. *Right click on the newly imported file and select "Validate" or click the  icon to validate the .CMF file.*

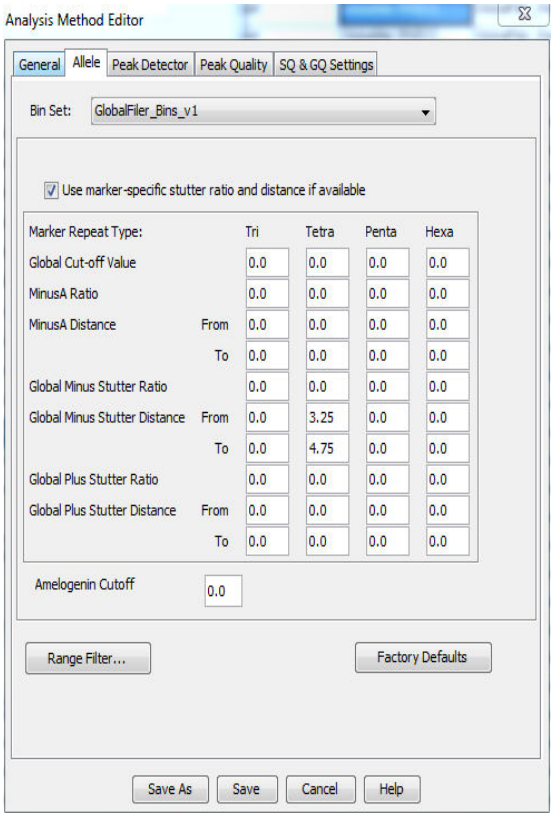
14. *Double Click on the newly imported file or click the*  *to execute the .CMF file.*
15. *Click on the "Import Reports" category in Message Center.*
16. *Double click on the new report, bolded in black, to view the Import Reconciliation Report.*
17. *Review any problems with the .CMF and verify the number of new specimens entered.*
18. *You can now pull up the newly added profiles in CODIS. They can be printed to PDF and saved for technical review and/or imported into Popstats.*

18.16 Analyzing Globalfiler with Genemapper IDX v1.4 software

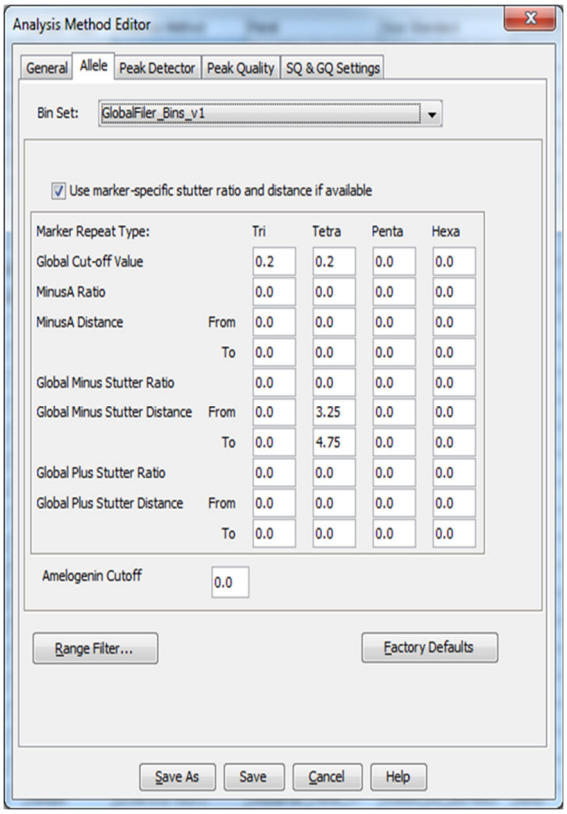
1. Follow the naming convention procedure for projects of (mmddyy.gf/iq/manual.initials/3500#.run date).

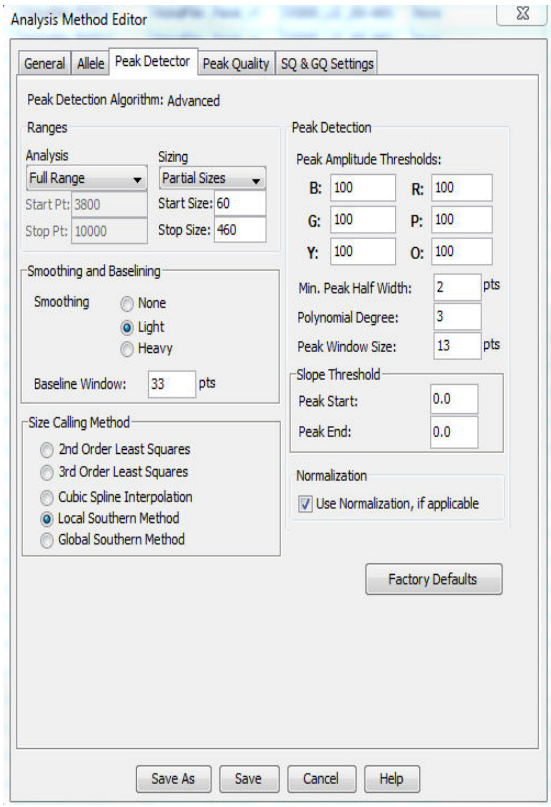
2. Globalfiler Analysis settings:
   a. Evidence
   Analysis method: Globalfiler 100 RFU
   Panel: Globalfiler_Panel_v1-dup
   Size Standard: GS600_LIZ_(60-460)
   Bin Set: Globalfiler_Bins_v1
   Stutter ratio: Marker specific (specific stutter values are loaded into the Panel manager)
   Global Cut-off Value: 0.0 for all Marker Repeat Types
   b. Reference Standards
   Analysis method: Standards GF 20% Cut-off 100 RFU
   Panel: Globalfiler_Panel_v1-dup
   Size Standard: GS600_LIZ_(60-460)
   Bin Set: Globalfiler_Bins_v1
   Stutter ratio: Marker specific (specific stutter values are loaded into the Panel manager)
   Global Cut-off Value: 0.2 for the Tri and Tetra Marker Repeat Types and 0.0 for the others
3. The screenshots below demonstrate the correct settings within the Analysis method.
4. Turn on the marker-specific stutter filters for N-2, N-3, N-4, N+3 and N+4.
5. The analysis range can be changed from "full range" to a more "partial range" to avoid the primer front and/or if sizing issues arise with the GS600 LIZ. The first allele bin is located after the 60 bp peak and is located at D2S441. Sizing needs to include the 60 bp peak as well as all of the other LIZ peaks through 460 bp to ensure correct sizing results.

Evidence Allele Tab



Reference Standards Allele Tab





19 Automated Quantitation, Normalization, PCR Set-up and CE Set-up for Globalfiler and Y-Filer Plus Evidence Samples

19.1 Quantifiler Trio Set-Up

1. Open EVOware Standard software.
2. Choose “Run an existing script” or “Edit an existing script”.
3. Select Favorites folder and the quantitation script i.e., “Air_Quantifiler_Trio”
4. Select “Run”. Select “Run” for the next prompt, also.
5. Enter number of samples to be quantified (include MB in calculation). Volume amounts of reagents will vary depending on number of samples entered to be quantified.
6. Choose the option of preparing the Master Mix manually or allowing the robot to prepare it.
7. Choose the option of preparing the standards or using previously made standards.
8. Follow screen prompts.
9. Tecan EVO 150 will begin processing
10. Once processing is complete, seal the sample plate with an optical adhesive cover.
11. Briefly spin sample plate.
12. Add sample plate to Life Technologies 7500.

19.2 Normalization

1. Open EVOware Standard software and follow prompted instructions.
2. Choose “Run an existing script” or “Edit an existing script”.
3. Select Favorites folder and the normalization script i.e., “Air_Normalization”.
4. Select “Run”. Select “Run” for the next prompt, also.
5. Import calculations from spreadsheet.
6. Click “create robot norm” and “create robot amp” on the spreadsheet
7. Follow screen prompts.
8. Tecan EVO 150 will begin processing.
9. When sample transfer is complete, apply an adhesive seal and return to Te-Shake
10. Once processing is complete, briefly centrifuge the sample plate, remove the seal, and return the plate to the Te-shake.

19.3 Globalfiler Amplification

1. Open EVOware Standard software and follow prompted instructions.
2. Choose “Run an existing script” or “Edit an existing script”.
3. Select Favorites folder and the Globalfiler amplification script i.e., “AirLiha_Globalfiler”
4. Select “Run”. Select “Run” for the next prompt, also.
5. Click “create robot amp” on the spreadsheet
6. Enter number of samples to be amplified (include MB in calculation but do not include the positive and negative controls). Volume amounts of reagents will vary depending on number of samples entered to be amplified.
7. Follow screen prompts.
8. Tecan EVO 150 will begin processing.
9. Once processing is complete, seal the sample plate with an adhesive thermal seal.
10. Briefly centrifuge sample plate.
11. Place sample plate on the Proflex thermal cycler.

19.4 Y-filer plus Amplification

1. Proceed as with Globalfiler through quantification, normalization, and Globalfiler amplification. For Y-filer plus amplification of reference standards with DNA IQ extraction, perform normalization based on quantification results then proceed.
2. Open EVOware Standard software.
3. Choose “Run an existing script” or “Edit an existing script”.
4. Select Favorites folder and the Yfiler plus amplification script i.e., “AirLiha_YfilerPlus”
5. Enter number of samples to be amplified. Include MBs but NOT positive and negative amplification controls. The volume of reagents depends on number of samples entered.
6. Select t (tubes) for Y evidence amplification.
7. Select p (plate) for Y standards amplification.
8. Follow screen prompts.
9. Tecan EVO 150 will begin processing.
10. Once processing is complete, seal the sample plate with an adhesive thermal seal.
11. Briefly centrifuge sample plate.
12. Place sample plate on the Proflex thermal cycler.
13. Setup CE plate as with Globalfiler

19.5 EVO 100 CE Setup

1. Open EVOware Standard software and follow prompted instructions.
2. Choose “run an existing script” or “Edit an existing script”.
3. Select CE setup script i.e., “CESetup_3500”.
4. Select “Run”. Select “Run” for the next prompt, also.
5. Enter number of samples to be setup, including MB. Volume of reagents depends on number of samples.
6. Follow screen prompts
7. Tecan EVO 100 will begin processing.
8. Once processing is complete, seal the sample plate with a plate septa. Briefly spin sample plate.
9. Place the plate with septa in a plate base and plate retainer.
10. Add plate to the Genetic Analyzer’s auto sampler.

20 Automated Globalfiler and Y-Filer Plus Testing of Standards

20.1 DNA IQ Non-Organic Extraction, Quantitation, Normalization, PCR Set-up and CE Set-up for Globalfiler and Y-Filer Plus Standards

20.1.1 Tecan EVO 150 Extraction (DNA IQ Non-Organic)

1. Remove ~¼ of a swab or ~3mm^2 cutting of stain and place into a 2.0 mL tube inside basket.
2. Prepare a manipulation blank at this time and label as **MB Date Initials**
3. Add 250 µL of prepared lysis/DTT buffer to each tube.
4. Incubate at 70^{o}C for 30 minutes.
5. Centrifuge at maximum speed for 3 minutes to remove lysis/DTT buffer from the cutting. Discard the cutting.
6. Open EVOware Standard software and follow prompted instructions.
7. Choose “Run an existing script” or “Edit an existing script”.

8. Select Favorites folder and the DNA IQ script i.e., “Air_DNAIQ8Tip”.
9. Select “Run”. Select “Run” for the next prompt, also.
10. Enter number of samples to be extracted (include MB in calculation). Volume amounts of reagents will vary depending on number of samples entered to be extracted.
11. Enter up to a 100 µL for elution volume.
12. Follow screen prompts. Tecan EVO 150 will begin processing.

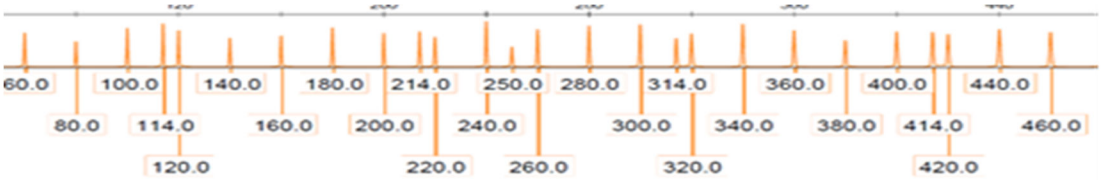
Proceed with quantitation, normalization and amplification scripts for Globalfiler or Y-Filer Plus

21 Interpretation of Globalfiler Results

Wherever feasible, interpret unknown samples prior to known reference standards.

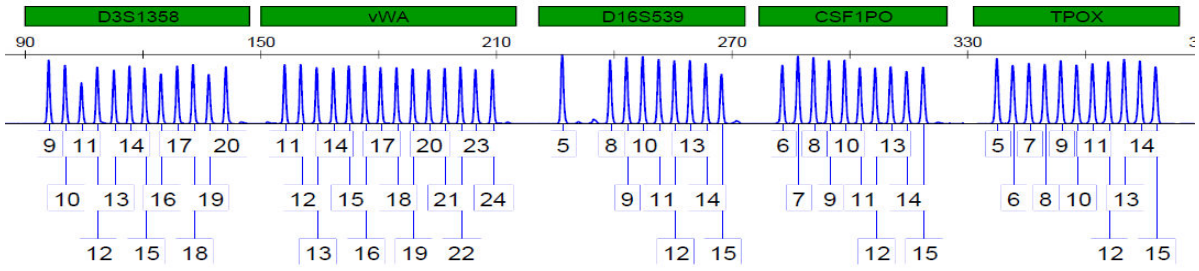
21.1 Assessment of Internal Lane Standard

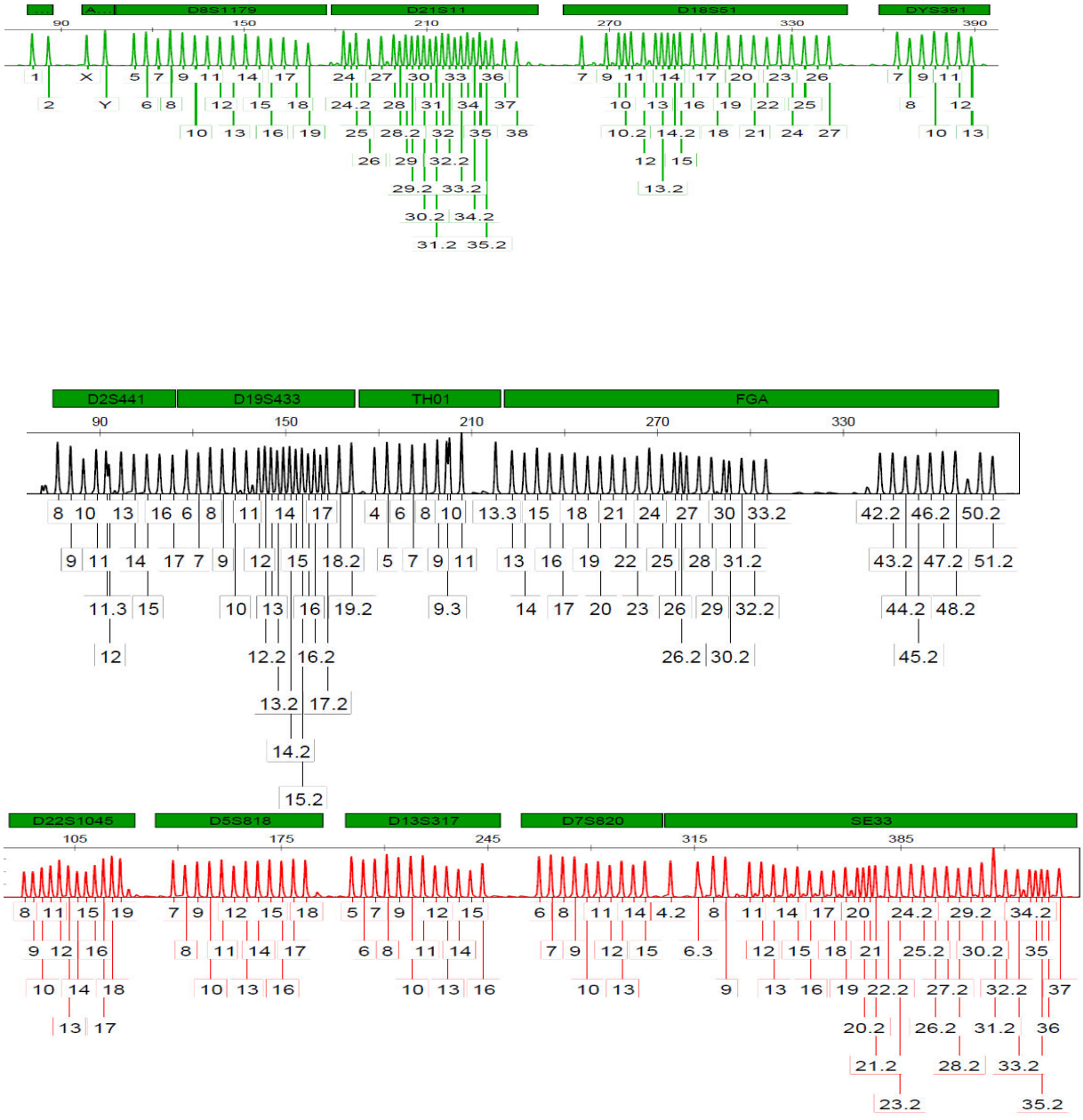
The LIZ 600 internal size standard must have the expected size results for the 60.0, 80.0, 100.0, 114.0, 120.0, 140.0, 160.0, 180.0, 200.0, 214.0, 220.0, 240.0, 250.0, 260.0, 280.0, 300.0, 314.0, 320.0, 340.0, 360.0, 380.0, 400.0, 414.0, 420.0, 440.0, and 460.0 base pair fragments. The peaks must be sizable but do not have to be above 100 RFU.

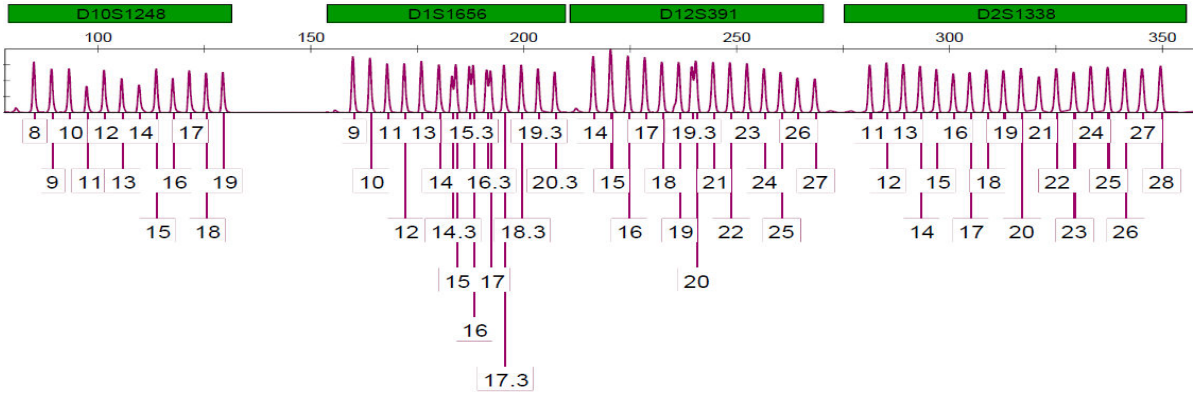


21.2 Assessment of Allelic Ladder

Examine the allelic ladders to determine that the software has assigned all allele designations correctly. The following alleles at the specific loci must be labeled and are at least 100 RFU.

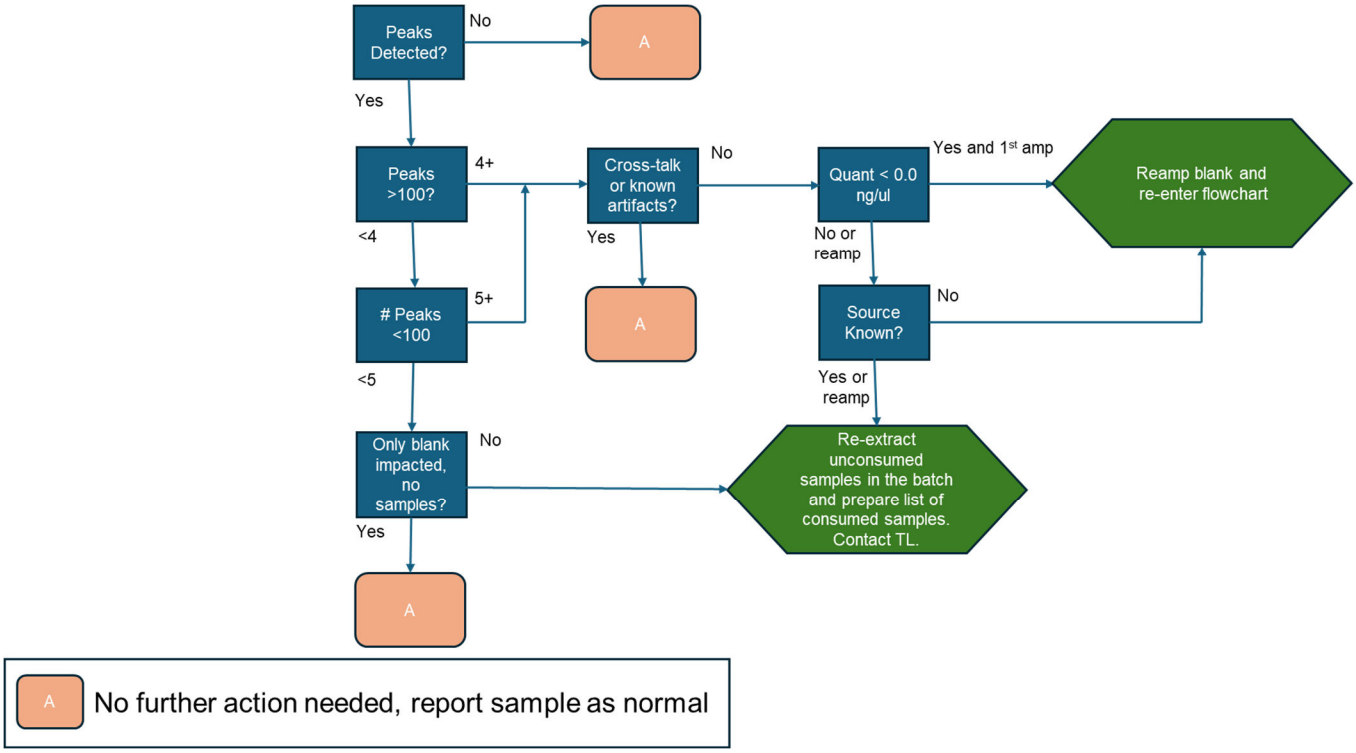



D8S1179
D21S11
D18S51
DYS391
D2S441
D19S433
TH01
FGA
D22S1045
D5S818
D13S317
D7S820
SE33



21.3 Assessment of Controls

1. Amplification negatives and manipulation blanks should contain no profiles. When only a single spurious peak, peaks consistent with cross-talk, or known artifacts are present, no further troubleshooting is required and data analysis may be performed. Where peaks above the 100 RFU analytical threshold are present, trouble-shoot as follows:
    a. For a negative amplification blank, re-prepare and run again. If the peaks are still present, consult the TL.
    b. For a manipulation blank, follow the chart below. Consult with the TL if needed.



2. Bring the troubleshooting results and proposed remediation and preventative actions to the TL. Potential courses of action include re-extraction, re-amplification, re-injection, interpretation of a reduced number of loci or reporting the sample as inconclusive. Enter all incidents into the inquiry log and keep documentation in the case file.
3. Amplification positives (either the kit positive or the NIST-traceable standard may be used) should contain the profile as given in the table below. If the profile is different, consult DNA management for troubleshooting assistance. Rarely, positive controls will fail to give any profile. Consult TL to identify if a secondary positive control can be designated.
4. Amplification Positive Control Types. Discuss with the Technical Leader if a NIST-traceable standard will be used for the positive control.

Globalfiler	Kit Positive 007
D3S1358	15, 16
vWA	14, 16
D16S1539	9, 10
CSF1PO	11, 12
TPOX	8
Y-Indel	2
Amel.	X, Y
D8S117	12, 13
D21S11	28, 31
D18S51	12, 15
DYS391	11
D2S441	14, 15
D19S433	14, 15
TH01	7,9.3
FGA	24, 26
D22S1045	11, 16
D5S818	11
D13S317	11
D7S820	7, 12
SE33	17, 25.2
D10S1248	12, 15
D1S1656	13, 16
D12S391	18, 19
D2S1338	20, 23

21.4 Assessment of Peaks

Peaks should fall within 100 to 30,000 RFU. Peaks below 100 RFU are below reporting standards. Those above 30,000 RFU and their associated stutter should be interpreted with caution. Consider diluting the amp product and re-injecting. A peak with good resolution is one with smooth sides and a sharp tip without excessive –A shouldering. Shoulders may be clicked off. The peak should originate from and return to the baseline, except in the case of peaks with only one base difference. Good background is a relatively flat baseline without

excessive noise or spikes. An analytical threshold of 100 RFUs and a stochastic threshold of 600 RFUs should be used for interpretation.

21.5 Artifacts

1. **Pull-Up**. Examine each sample for any small, labeled peaks present that approximately line up with a relatively large peak in a separate dye. This may indicate fluorescent pull-up. If Genemapper ID-X labeled the peak, click it off and label only with "pull-up" or "PU".
2. **Spikes**. Examine each sample for extraneous labeled peaks present in two or more dyes at the same relative base pair. Click off and label only with "spike".
3. **Stutter**. N-2 (SE33 and D1 only) N-3, N-4, and N+3 (D22 only) filters are on for casework evidence samples. Additional forward stutter filters are typically used. IDX does not label peaks in stutter positions within the pre-set averages. The stutter filter is a labor-saving device that may be overruled as needed. Analysts should consider RFU values to determine if forward stutter peaks should be labeled as true alleles. Reference samples may be analyzed with the Analysis Method Editor Allele settings at 0.2 for the Global Cut-off Value at the Tri and Tetra marker repeat types.

Globalfiler N-2, N-3, N-4 Stutter Filter Values					
D3S1358	**vWA**	**D16S539**	**CSF1PO**	**TPOX**	
10.98	10.73	9.48	8.77	5.55	
D8S1179	**D21S11**	**D18S51**	**DYS391**		
9.60	10.45	12.42	7.43		
D2S441	**D19S433**	**TH01**	**FGA**		
8.10	9.97	4.45	11.55		
D22S1045 (n-3)	**D5S818**	**D13S317**	**D7S820**	**SE33**	**SE33 (n-2)**
16.26	9.16	9.19	8.32	14.49	3.97
D10S1248	**D1S1656**	**D1S1656 (n-2)**	**D12S391**	**D2S1338**	
11.46	12.21	2.45	13.66	11.73	
Globalfiler N+2, N+3, N+4 Stutter Filter Values					
D3S1358	**vWA**	**D16S539**	**CSF1PO**	**TPOX**	
5.20	5.80	5.20	3.00	n/a	
D8S1179	**D21S11**	**D18S51**	**DYS391**		
3.90	4.80	9.90	7.60		
D2S441	**D19S433**	**TH01**	**FGA**		
11.70	6.10	n/a	9.40		
D22S1045 (n+3)	**D5S818**	**D13S317**	**D7S820**	**SE33**	**SE33 (n+2)**
6.69	3.90	5.50	n/a	6.00	n/a
D10S1248	**D1S1656**	**D1S1656 (n+2)**	**D12S391**	**D2S1338**	
5.40	4.80	N/A	6.10	9.70	

Values represent the mean plus three standard deviations.

4. **Off-ladder alleles**. Allele calls are made by comparing the size of the DNA fragments in samples to the size of the fragments in the allelic ladder. IDX labels many virtual alleles as well. Peaks not consistent with ladder peaks or virtual alleles are labeled as off-ladder by IDX. If the sample data is sufficient for comparison, resolve these by re-injecting the sample or by its presence in multiple samples. If the re-injection again sizes as an off-ladder allele, the dye containing the OL for each sample injection along with the ladder used for analysis should be zoomed in to the corresponding locus, labeled with the base pairs and printed together on the same page. The allele call is determined by using the base pair size of the closest ladder allele compared to the base pair size of the off ladder allele. Show the calculations on the zoomed-in printout. Designate the off- ladder as follows: x.1, x.2, or x.3, where x is the number of full repeats and the decimal represents the number of additional bases. If the off-ladder occurs outside the range for the ladder alleles for that locus, designate the off-ladder as greater or less than the closest allele.

5. **Known Globalfiler Artifacts**. Artifacts referenced in manufacturer documentation can be clicked off with no label required.

6. **Tri-allele.** Rarely, a sample containing three alleles at one locus may be from a single source (triallelism). Verify tri-alleles by re-injecting the sample or by its presence in multiple samples.

7. **Cross-Talk**. Cross-talk may occur between capillaries in an array when a sample with very high RFU values is injected adjacent to a capillary containing little or no DNA. The cross-talk data is typically observed in samples as off-ladder peaks with a similar pattern to the high RFU sample. When necessary if interpretation may be affected, samples with suspected cross-talk should be re-injected separately from the strong sample.

8. **Carry-over**. Carry-over is the physical transfer of DNA from one injection to the next. The wash between injections should clear the capillary and tip prior to entering the septa for the next sample. The evaluation of carry-over within a capillary is performed evaluating the subsequent injection for any sign of signal from a previous injection. These will typically be unlabeled peaks with the same pattern as the previous sample. This may be observed with allelic ladder injections or samples with high RFUs.

21.6 Allele Call Sheet Guidelines – Reference Standards

Note: Allele calls for each locus will be documented on electropherograms for evidence samples and no longer included on a separate Allele Call Sheet (ACS). Artifacts will be unlabeled and/or crossed-out on each electropherogram so that only true allele calls are labeled. The alleles for use in statistical calculations will be documented on the electropherogram, except for complete single source samples. The alleles labeled on the electropherogram can be used for complete single source statistical calculations. The alleles for expected contributors and data not sufficient for comparison do not have to be retyped on electropherograms.

1. Complete separate result charts for case reference samples. The positive, negative, and manipulation blank will not be included on the allele call sheet.
2. If no results were detected for a particular locus or sample, note in the chart not detected by writing ND. Writing ND once followed by a long arrow through the remaining locus boxes is sufficient.
3. If reporting a sample as data not sufficient for comparison, note on the electropherogram "DNSFC" with the reason. Writing "DNSFC" once is sufficient. These reference samples do not need to be included on the ACS.

21.7 Directions for Auto Populating a Globalfiler Call Sheet for Reference Standards.

1. Open a Globalfiler project.
2. In "Table Setting" in the ID-X toolbar, select "GF Auto Call Sheet export".
3. Navigate to the "Genotypes tab" and it should display the following: Sample File, Sample Name, Marker, Dye, Allele 1, Height 1, etc…
4. Go to File and click on "export Combined Table".
5. In the new window, type a file name or leave as is and select a destination to save the text file.
6. In this new window, in "Export File As", select "tab-delimited text (.txt)".
7. In "Merge" box, select "One line per marker" and check "Include all marker Information".
8. Click export.
9. Open the Allele Call Sheet file.
10. Click on "Import Data" in the "Start Here" tab.
11. Navigate to the location where the text file was saved.
12. Click on the file name so it populates the "File Name" box. Click open.
13. All cases should show as individual tabs within the excel workbook and all fields populated with the injection (1 or 2) optional.
14. Click "save as" and save the file as the batch folder name.

21.8 Interpretation Documentation

Interpretation documenting the thought process used in the interpretation of profiles will be included on the electropherogram. Documentation of interpretation should capture details so that another scientist will understand the thought process to include number of contributors, number of major contributors, degradation on the right hand side, and/or one contributor degrading versus another, etc.

Information on the electropherogram:

- All alleles that are labeled/not crossed-out on electropherograms are considered true alleles.
- Stutter alleles and artifacts will either not show on the electropherogram or be crossed out with a reason stated (Pull-up, etc.).
- Alleles that would be used for stats must be listed. This includes majors, minors, and mixture stats.

- Expected/assumed contributor alleles can be labeled with dots or otherwise to aid in easy recognition. This would supplement the available profile of the expected/assumed contributor.
- Clear single source profiles do not need the alleles retyped. No additional alleles can be labeled on the electropherogram at the loci to be used in the SS stat. For partial profiles, an X with reason (see below) will be listed for unused loci.
- If the sample is reported as single source with a few low extra alleles as allowed in the manual (section 22.3), only the additional <5 alleles would be listed below the locus in ( ). The alleles not listed in ( ) under the locus would be used in the stat. ( ) indicate low level additional alleles.
- Entire electropherograms reported as DNSFC do not need alleles types below loci, but as stated above, the labeled alleles must reflect the characteristics (alleles) that are identified as "true" for the loci.
- Loci not included in the stat will be marked with an X and reason noted such as <600 and or >40%.
- When peak height ratios are near the 40% or 60% values impacting interpretation, the alleles for the ratio will be identified and the % listed. This will aid reviewers and provide good documentation.

General Guidance:

- Assessment of loci for use in potential statistical calculations will be made prior to comparison to known standards. It is acknowledged that some stats may be modified for scientifically justified reasons after comparison with known standards. Impacted loci likely would be excluded from the issued stat but may not in all situations.
- The alleles for statistics (including "any" and obligate designations) must be documented except for complete single source profiles.
- A reason for not using loci in statistics will be documented. This could be due to degradation, drop-out or potential drop-out, indistinguishable major/minor, potentially stochastic data, etc.
- Multiple statistics may be issued for a profile; however, only one CPI mixture statistic can be determined. For example, you can have a single source major and general mixture stat. A minor RMP stat may be issued as well.
- How interpretation is documented is intended to be flexible but the assumptions used must be stated on the form or the electropherogram.
- Enter all forensic sample profiles into LDIS as permitted by CODIS guidelines. In a case where several samples yield the same profile, only one of the samples needs to be entered.
- Enter suspect known samples into LDIS if no suitable matching forensic profiles are found.

22 Globalfiler Interpretation and Statistical Calculations.

22.1 Determine if the e-gram is interpretable

1. Use 100 RFU as the analytical threshold and 600 RFU as the stochastic threshold.
   Major profiles can be calculated where the peak height ratio of the highest minor contributor(s) allele to the lowest major allele is less than 40%. The peak height ratio for single source major contributors should have heterozygous pairs >60% and >600 RFUs. Homozygous alleles for the major should be at least 1200 RFUs and 4 times the RFU value of the highest minor allele. A minimum of 5 interpretable loci from a two person mixture is required. A minimum of 10

interpretable loci are required to report a three person mixture (1 or 2 major/3 contributors) or a major part of a four person mixture (1, 2, or 3 major contributors). Only a single source major may be reported for a sample with clearly five contributors. No interpretation for mixtures with greater than five contributors should be made.

2. If a sample is highly degraded but the smaller loci demonstrate strong data and 5/10 loci are not interpretable, fewer loci may be used based on discussion between the reporting analyst and technical reviewer.
3. The above guidelines apply to samples with reasonable DNA condition and quantity. Look at the e-gram as a whole. If peaks are present at only 5 loci, be aware that otherwise interpretable loci may be affected by allelic drop out or elevated stutter. Quantifiler Trio values correlate well with e-gram appearance but are still subject to some variation. Per-contributor targets below 0.25 ng are more likely to be affected.
4. TARGET > 0.25 ng = good, keep in mind the proportion of contributors in mixtures. ≥0.75 ng generally needed for usable three person and higher mixtures.
   TARGET < 0.25 ng = stochastic

   DI = 2 predicts some degradation but take no action.
   DI = ≥ 3 may benefit from increased target

   If quant > 5 ng/µl, target 2 ng. Higher target will make left loci blow out. If quant less than 5 ng/µl, target ~3 ng.
   If large amplicon not present AND IPC normal, amplify >1.0ng.
5. Interpretable samples with three or four contributors will generally have 0.75 ng or greater DNA input. In rare instances, the RFU values of a sample may indicate the quant value varied from the true concentration. Interpret with caution and ensure proper documentation of interpretation.
6. **If >3 contributors and target <0.75 ng**, most likely “Data not suitable for comparison”.
   If 2-3 contributors, estimate each person's relative portion of the target.
   Use caution when interpreting major mixtures when ~2ng is targeted.

22.2 Stutter

1. For single source profiles with low quant targets, use caution when peaks are in stutter positions.
2. When first evaluating the profile, take RFU values for what they are. Locus specific stutter has been applied with called alleles higher than the stutter percentage. Stutter RFUs are not subtracted from the listed RFU values.
3. If remaining allele RFUs may be close to 600 after stutter filter application or to review if a rule is violated, then apply and subtract for stutter from right to left across a locus.
4. For single source and single source major profiles, evaluate adjacent alleles as called with GeneMapper. Do not subtract stutter RFUs from adjacent alleles in single source or major adjacent alleles for the single source donor in mixture.
5. In general, do not consider stutter when calculating for the 40% Rule. There is one exception:
   i. When there are the max number of alleles for a major contributor or major group of contributors, it is appropriate to calculate stutter to determine if the 40% rule can be met.

6. When evaluating mixture statistics with peaks close to 600 RFU and in stutter positions, calculate stutter. If the peak drops below 600 RFU, consider application of allele, any or leave this locus out of the statistical calculation. This will be situation dependent.
7. Forward stutter will generally only be calculated when there are very high RFUs (>10k).

22.3 Determine if single source or mixture

Single source if no indication of second contributor outside of stutter positions. For low level samples, be mindful of unlabeled peaks near baseline and elevated stutter. 5 or more peaks in non-stutter locations = mixture.
For mixture, determine number of contributors
Number of contributors = greatest number of labeled and unlabeled peaks at any locus/2. Include unlabeled real peaks. Genotype of assumed contributors and peak heights may assist.

1. A profile is the sum total of all of the alleles at all of the loci tested. It may represent DNA from a single individual or from multiple individuals.
2. A single source profile is one where there are no more than two peaks at each autosomal locus, with the exception of occasional tri-allelic or trisomic samples.
3. In a single source sample, heterozygote peaks heights are generally within 60% of each other. Exceptions may occur due to very low DNA quantity causing stochastic artifacts, degradation where the larger allele is weaker, or primer site mutations.
4. Where extra peaks occur, multiple contributors may be present. A single unattributed peak should not be interpreted as an additional contributor. Rather, the entire multi-locus profile must be taken into consideration, and multiple indications of an additional contributor should be present. 5 or more additional peaks that are not in forward or reverse stutter positions is indicative of a mixture. Consider peaks above the analytical threshold and those below distinct from baseline.
5. Where data is ambiguous, the determination of single- or multiple-source profile is best made after a careful review of DNA quantity and condition and all locus data.
6. When there is a known or assumed contributor, treat the mixture as the number of contributors minus 1.

22.4 Determine pattern of contributors

Based on entire e-gram,
single source
unresolvable
major/minor
removable known contributor
major mixture/minor
If no major, then a mixture stat if possible**.**

22.5 Type of calculation

The allele frequencies loaded into the CODIS Popstats and ArmedXpert program may be used to calculate profile frequencies. The minimum allele frequency used with Popstats and ArmedXpert is

5/2n for each population database, where n is the number of contributors in the database for the given population.

Random Match Probabilities (RMPs)

If an individual is included as a possible source of the biological specimen, then a random match probability may be determined. RMPs are calculated with the Popstats or ArmedXpert software for single source samples. ArmedXpert may be used for RMPs associated with mixed samples. A frequency may be calculated at one or more loci for a profile.

The following Popstats and ArmedXpert equations are recommended by the National Academy of Sciences National Research Council for use in RMP calculations:

Heterozygotes: 2pq where p and q are the individual allele frequencies.
Homozygotes: $p^2 + p(1-p)(\Theta)$ where $\Theta = 0.01$

Allele, Any: $p^2 + p(1-p)(\Theta) + 2p(1-p)$

The product rule is used to calculate the combined profile frequency. Calculations for all available major racial groups should be made.

Combined Probability of Inclusion (CPI) Statistics with Mixtures

The frequency of the population that CANNOT be excluded as potential contributors to mixtures or the major sources of mixtures can be determined with the CPI/rCPI. Popstats uses the formula $(P_1 + P_2 + P_N)^2$ where P1 is the frequency of the first allele, etc. to calculate the frequency for each locus of the mixture. A theta value is included for the calculation. The product rule is then used to determine the overall combined probability of inclusion (CPI). A restricted CPI may be applied to multiple major contributors despite the presence of minor contributor(s) alleles as described below.

22.6 Determine type of calculation

CPI-no assumptions, use all loci lacking peaks under 600 RFU
rCPI - with number of contributor assumption
RMP (Random match probability) – Standard statistic
uRMP (unrestricted RMP) – no concern for drop-out, make assumptions based on # of contributors
rRMP (restricted RMP) – can possibly deconvolute into contributors, allele combinations considered
mRMP (modified RMP) – modified with use of “allele, any” where drop-out is considered
fmRMP (forced modified RMP) – use of “allele, any” with data above 600 RFUs

22.7 Profile interpretation minimums

22.7.1

To promote uniformity in the interpretation of low-RFU data, minimum data requirements have been devised that take into consideration the stochastic amplification expected at low template levels as well as experience with various substrates.

	Single Source	Single Source Major	2p Mixture OR 3p Mixture	Single Source Major	2p Major Mix, 3p Major Mix
Total Contributors	1	2	2 OR 3	3, 4, 5	Up to 4
Het Peak Height Min	≥ 100 RFU	≥ 600 RFU	≥ 600 RFU (consider stutter)	≥ 600 RFU	≥ 600 RFU (consider stutter)
Het PHR Min		≥ 60%		≥ 60%	
Hom Rules	600 RFU	≥ 1200 RFU ≥ 4x largest minor peak	≥ 1200 RFU	≥ 1200 RFU ≥ 4x largest minor peak	≥ 1200 RFU ≥ 4x largest minor peak
40% Rule		YES		YES	YES
Min # Interpretable Loci	≥ 1	5	2p Mixture = 5 3p Mixture = 10	10	10
AX Use	*	**	***		***

See Appendix for specific examples.

*For singular alleles between 100 and 600 RFU, apply "allele, any"

Due to possible sharing/allele overlap, the overall data should be considered in addition to the identification of a minimum number of loci for a statistic.

**For a single source major, with a small amount of a second person in AX, you can:

- Apply "allele, any" to homs that are 600 – 1200 RFU, but meet the 4x rule.
- Apply "allele, any" to singular alleles (only one allele called at the locus) 600 – 1200 RFU where it is assumed the 2nd person has dropped out.
- One het allele over 600 RFU, one het < 600 RFU – apply "allele, any" to the allele > 600RFU.
- Two het alleles <600 RFU, leave locus out
- Do not apply this approach in profiles with lower ng targets, or if multiple minors are present.

***Two, Three, or Four, Person Mixtures:

- When the maximum number of alleles are present for the assumed number of contributors (2 person example, 4 peaks called), they all need to be > 100 RFU after stutter considered.
- When there are the maximum number of alleles at a location for the number of contributors included in the statistic, consider only heterozygous combinations. Profile qualifications:
    - Applies to 2p, 3p, 2p major mix, and 3p major mix
        - For a major mixture, apply stutter to ensure all alleles considered in the statistic remain > 600 RFU.
    - Quant target generally >0.75ng. Use caution if below.
- For two-person and three-person mixtures, when there are the maximum number of alleles minus 1 (n-1), and **one** allele drops below 600 RFU, apply "allele, any" to the allele that drops below and choose other alleles.
    - Thought is you are possibly only missing one allele, considering "allele, any" for the allele that drops below 600 RFU will cover the lost allele.
- When you are using the expected contributor to deduce in a two-person mixture, treat the remainder as a single source profile.
    - PHR of remaining het peaks does not necessarily need to be 60%. Your assumption of two total contributors trumps the PH imbalance.

22.7.2

The following guidance to support minor data being from a single contributor emphasizes evidence typically submitted in sexual assaults. For other types of evidence, consider the intimate criteria when items would typically have DNA from a limited number of contributors.

Intimate samples: Intimate defined as orifice and skin swabs from area usually covered by clothing.

- If at least 3 heterozygous loci are indicated with data above or below AT and no indication of an additional minor contributor, a stat for the minor may be issued. Only one of the three loci with het minor must be labeled. Non-stutter heterozygotes are ideal. Baseline noise may impact confidence in some minor labeled peaks, interpret with caution.
- Otherwise report minor as additional DNFSC.
- Y-STR may still be recommended for partial minor data reported as DNSFC.

Non-intimate samples:

- If at least 3 heterozygous loci with ≥600 RFUs (after stutter evaluation) are present, a stat for the minor may be issued. The rest of the minor data used in the stat can be 100 RFU or higher.
- Otherwise report minor as additional DNSFC.
- Y-STR may still be recommended for partial minor data reported as DNSFC.

22.8 Troubleshooting for weak e-grams

- Weigh the costs/benefits of producing a stronger e-gram.
- Inhibition may be resolved by stock dilution or clean-up steps.
- **DI = 2** predicts some degradation
- **DI ≥ 3 may** benefit from amplifications of increased target
- Ideal target = 1 ng generally giving 4000-8000 RFU heterozygote peaks at D3S1358.
- Is e-gram appearance consistent with quant data?
- Per-person targets under 0.25 ng expect stochastic behavior, e.g. caution with 3-person mixture under 0.75 ng

22.9 Deconvoluting overlapping contributors

Shared alleles in 2-person mixtures or major mixtures may be deconvoluted using proportion estimates informed by other loci and applying peak height ratio expectations. Due to heterozygote peak height variability, a potentially shared minor allele may not be deconvoluted in a major/minor mixture locus.

22.10 Multiple calculations on the same e-gram.

- major and minor contributors
- single source major and mixture
- major(s) and mixture
- Do not perform more than 1 CPI/rCPI mixture calculation on an e-gram.

22.11 General guidelines

22.11.1 When to calculate or report a statistic

Do not calculate a statistic if the presence of DNA from a victim, consent partner, or elimination standard is reasonably expected and not probative.

Examples –

- A vaginal swab or underwear match to the victim
- A vaginal swab or underwear match to the victim's consensual partner
- Resident or owner match to items from residence, vehicle, etc.

Do calculate a statistic for each evidence profile where a victim, elimination, or suspect is included and considered probative.

Example --

- A vaginal swab match to a suspect
- Knife blood stain match to a victim

A qualitative statement similar to the following must be used if a statistic is not provided.
Victim – expected contributor
John Doe – consent partner
John Doe – owner of [Item]

22.11.2 Multiple stains have been tested

a. Calculate a statistic for each stain individually if different profiles.
b. All identical profiles for the same stats entry can be lumped together for a single calculation.

- Seven blood stains at a B&E scene match the subject. All are complete profiles. All items would be lumped together in one calculation for reporting in the DNA Conclusions.
- Five blood stains on the victim's clothing match the suspect, five stains from the window at the scene match the suspect and five blood stains on the suspect's clothing match the victim. Each profile is different for statistic entry. Calculations for all inclusions are needed: each profile from the window, each profile from the victim's clothing and each profile from the suspect's clothing.

22.11.3 Documenting assumptions and other interpretation decisions.

When an assumption is made in the interpretation of a profile, document that assumption in the case file and at a macro level in the report. For the case file, the e-gram or the interpretation worksheet are available for use.

a. Number of contributors
b. Expected contributors

c. Interpretation approach
d. Reasons for not using a locus in the calculation
e. Calculate a statistic for all e-grams except complete single source or complete single source major.

22.11.4 Reporting of statistics

a. Calculations using the NIST population database are performed for casework profiles from Identifiler and Globalfiler testing. The FBI amended population database may be used for supplemental reports of previously reported statistical estimates made with the original FBI database.
b. Report based on only the most common of the 3 default figures generated.
c. Calculations rarer than 1 in 1 trillion are reported as "rarer than 1 in 1 trillion."
d. Calculations more common than 1 in 1 trillion are reported in tiers by rounding down to the first digit (e.g., 100, 500, 1000, 5000, 10,000, 50,000, 100,000, etc.).
e. Samples with the same tiered statistic can be grouped together for reporting.
f. Report profiles more common than 1 in 300 as inconclusive.

22.11.5 Inclusions and exclusions--<u>Interpret/calculate a profile before examining the reference standards</u>

If an evidence profile is different from that of a reference standard, then that individual is excluded as the source of the biological specimen. An exclusion is independent of the frequency of the profile in the population. With the exception of alleles lost due to degradation or dropout, if the results for only one locus do not match, exclusion is determined.

If an evidence profile is the same as that of a reference standard, then that individual is included as a possible source of the biological specimen. A frequency may be calculated at one or more loci for a single source profile.

A mixture inclusion requires:
The presence of the same alleles as found in standard at some or all of the loci in the evidence sample.
Where the profile is incomplete, the pattern of inclusive loci must make sense in terms of the relative performance of the loci. The strongest performing loci must contain alleles consistent with the standard.
The exact number of consistent loci/alleles required to make an inclusion is dependent upon the quality of the profile—number of contributors, presence of inhibition, presence of degradation, etc.
A mixture exclusion requires the strongest performing loci to lack alleles consistent with standard.
An inconclusive may be called where the presence of multiple contributors, low DNA quantity, degradation, and/or inhibition result in a poor profile.

22.11.6 Composite Profiles

Partial DNA profiles resulting from multiple amplifications and/or injections of the same DNA extract may be reported as a composite DNA profile. If there is a reasonable expectation that the samples originated from a common source (e.g. multiple swabs collected from the same orifice or multiple samples from the same bone) a composite DNA profile may be reported.

22.11.7 Comparisons to Data from other Sources

When making comparisons between profiles generated using different systems (for example, Profiler Plus vs. Identifiler or Powerplex) or between profiles generated by different labs (for example, BCI vs. a private lab), use the data as reported, rather than re-interpreting. Data from the Miami Valley Regional Crime Lab (MVRCL) is an exception, follow Appendix B.

23 DNA Analysis in ID-X v1.4/ArmedXpert v3.0.8.27

1. Launch the software by clicking on the ArmedXpert icon- (double helix) on the desktop.
2. In the login screen, enter your CODIS user name and click okay.
3. Click on the top left double helix which accesses the main menu and shows a drop- down list.
4. Select Import→ Other. Navigate to the ID-X text file that was exported from ID-X, highlight the file and click “open”.
   To export a text file from ID-X:
   - Open GM ID-X v1.4.
   - Open the project and in the table settings in the top ribbon bar, select “ArmedXpert Export”.
   - Go to File and select “Export combined table”.
   - Name the txt file and navigate to where the file is to be saved. Make sure it is a txt file and one line per marker and click “Export”.
5. A table appears with all of the samples that are contained in the selected file.
6. To view all of the samples in the table again, click “Views” and select the “All” radio button on the top left.
7. To save the project, navigate to the double helix in the top left corner of the main AX window and select “save”. A new box will open asking to save the project, click “yes”. Navigate to where the project will be saved and name accordingly. This generates a “.axp” file and is the saved ArmedXpert Project.
8. To begin an interpretation, click on the “Interpretation” tab. On the left hand side of the ribbon, click “Begin Mixture Interpretation”. A new window will open referred to as the “Mixture Interpretation Window”.
9. Within the “Mixture Interpretation Window” there are three different ways to select the sample to be analyzed:
   a. Using the “Pick via mouse” button, click on the sample to be analyzed in the initial table of samples.

b. To the right of the “Pick via mouse” button there is a drop-down list. This list contains all of the samples imported. Choose the sample from this list to be analyzed.
c. To the right of the “Pick via mouse” button, you can type a portion of the sample name. This will sort the sample and give the filtered results in the drop down list. Use the computer mouse or arrow keys to select the sample.

10. When the sample is chosen, it will populate the “Mixture Interpretation Window”. ArmedXpert will then present possible allele combinations for the selected sample and allow the user to assign combinations to different contributors (i.e. major, minor).

    Note: Make sure to edit the number of contributors (top right corner of window).

11. Assigning allele combinations to contributors can be done in three ways:
    a. Select the name (i.e. major) in the drop-down box wand then select the alleles for the specified contributor. Then select the next name in the drop-down list (i.e. minor) and choose the other alleles to be assigned to the that contributor.
    b. Under the “Mixture Information” portion of the “Mixture Interpretation Window”, right click on the allele combination presented, then click “send to major” or “send to minor”.
    c. Use the “Popout Calls” button = Another window will open displaying all alleles called at a locus. The analyst can click the box of the corresponding alleles to be assigned to the major and minor contributors respectively. You can move to the next locus by clicking the arrow next to the locus name at the top of the “Popout calls” window.

       Note: within the “Mixture Interpretation Window” is a checkbox labeled “Highest to lowest #”. This will determine the order in which the loci are presented to the analyst. If checked, the loci with the most alleles called will be presented first. If this is not checked, the program will move through the profile starting at the top of the e-gram and working left to right.

12. Any allele combinations presented in the “Mixture Interpretation Window” with at least one allele below the stochastic threshold of 600 RFU will be highlighted in pink.
13. To determine what allele combinations presented by the program under the “Mixture Information” portion of the “Mixture Interpretation Window” should be considered, see Appendix A for additional guidelines. It may not be possible to assign a major and minor at every locus. For example, it may be possible to only deconvolute the major genotype.
14. Tools within the software allow for more than one allele combination to be considered for a given contributor. Using the obligate or “allele, any” functions will consider multiple allele combinations within the statistical calculation. Guidance on the use of these functions is provided within Appendix A.
    a. Obligate allele – will consider all allele combinations that include this allele

b. Allele, any – will allow for a homozygous allele combination, as well as all heterozygous allele combinations with the selected allele (the selected allele and all other known alleles at that locus).

15. Once all of the loci have been evaluated, the deconvolution can be viewed using the "View Call Report" button. The pop-out call window can be closed.
16. The "View Call Report" displays all alleles of the original profile and the allele combinations chosen for each of the deconvoluted contributors.
17. To calculate a statistic, navigate to the interpretation tab at the top of the main ArmedXpert window. Click "RMP" or "Single Source". This will generate a red highlight box. Navigate to the Allele Call Report and click the name of the sample on which to calculate the statistic.
    a. If calculating a single source statistic, a frequency table is generated with the calculation for three ethnic groups (African American, Caucasian, and Hispanic). To print this page, navigate to the main ArmedXpert window, click the double helix, select print and follow the prompts.
        i. Select these settings when printing: portrait, sheet, and fit to two pages.
    b. If calculating an RMP, a new window will open to show allele combinations for each locus chosen during the mixture interpretation.
        i. Prior to the main window opening, a small window will pop up and say, "Add all homozygous and heterozygous allele combinations" with buttons to click yes or no.
            1. Yes will calculate a CPI on the alleles chosen during the interpretation and will include Theta in the mathematical calculation.
            2. No will only generate the mathematical calculations for locations where two alleles were chosen. The analyst will then need to specify the allele combinations to be included at the other loci.
        ii. At this stage combinations can be added or removed. The statistic will be displayed at the bottom of the window and updates in real time as combinations are added or removed. At the bottom of the RMP window is a calculations tab which shows the mathematical calculation at each locus.
            1. To include Theta in the calculations, choose the specific hom or het combos, or "all homs" and "all hets".
        iii. To delete a combination, hover over the combination and double click
        iv. To add a combination, click "Add combo" and choose the combination or group of combinations to be added.
18. Close the RMP window. Saving the file is optional since it will be printed for the case file.
19. Upon closing the "Mixture Interpretation Window", a save box will open – "Save Changes to Mixture Interpretation Window-DDA Interp." click "yes". The interpretation itself will be saved within the ArmedXpert project and can be reopened.

a. To reopen: While in the general ArmedXpert project, navigate to the interpretation tab, click the organize button (right side), click the "+" sign next to "Windows", then select the appropriate "Mixture Interpretation" for the sample.

24 Kinship

24.1.1 Interpretation of Kinship Sets

The particular formulas used to calculate the locus Index values depend on the specific allele relationships in the kinships and are given in the Popstats supporting documentation. The overall profile Index is obtained by multiplying the individual locus Indexes together with the exception of vWA and D12S391.

The vWA and D12S391 loci are located on the p arm of chromosome 12. These loci were determined to be independent at the population level for calculation of RMP and CPI statistics. However, for kinship analysis/parentage calculations, the loci cannot be assumed to be independent. For kinship/parentage statistics, either the vWA or D12S391 locus can be included, but not both. As the default, the vWA locus should be used for these statistics. Be aware, there will be situations where vWA is not useful in discriminating the relationship of the individuals tested and the D12S391 locus should be used.

All loci are examined for common alleles consistent with inheritance. Due to the relatively high mutation rate of STR loci, three exclusions are required to conclude that the alleged parent is excluded. Popstats supports the calculation of one- and two-mutation trios. The default data in Popstats will routinely be used for these calculations.

24.1.2 Calculations for Paternity Test--Both Parental Reference Standards Present

The numerical value of a paternity match can be expressed in terms of the paternity index (PI), which is defined as the probability of the genetic observations if the alleged father is the true father divided by the probability of the genetic observation if the alleged father is not the true father.

Choose the paternity calculation option and enter the mother, alleged father and child profiles into the Popstats Program. The output gives the locus and overall profile parentage index (PI), probability of parentage (PP) and probability of exclusion (PE) for several population groups.

24.1.3 Calculations for Reverse Parentage

When evidence samples such as unidentified remains, crime scene evidence, or kidnapped/abandoned babies are submitted for comparison to samples from both biological parents, a reverse parentage calculation is performed. The reverse parentage calculation requires three DNA profiles: Biological Mother, Biological Father, and Alleged Child. In Popstats select the subtype "Reverse" for the parentage calculation and enter the profiles. The output gives the locus and overall profile reverse parentage index (RPI), and probability of exclusion (PE) of reverse paternity for the biological child for several population groups. The probability of exclusion of reverse parentage is the proportion of all individuals that do not contain an allele that matches the biological mother and a second allele that matches the biological father. This can also be stated as the probability that a person randomly selected from a population can be excluded from being the biological child of two known parents.

24.1.4 Calculations for Single Parentage—Only One Parental Reference Standard Present

If a complete trio for the kinship analysis is not possible, a single parentage or "motherless paternity" calculation can be performed. In Popstats select the Kinship option and the "PO" abbreviation for parent-offspring. The DNA profile from the child must be entered into the Reference side in Popstats and the mother or father into the Evidence side. For this purpose, it does not matter if the unknown sample for investigative purposes was from the parent or the child of the known reference sample submitted to the laboratory. Popstats will apply potential mutation calculations to the DNA profile entered as the reference sample for determining the probability of exclusion. The output gives the parent-offspring index and the probability of exclusion. The parent-offspring index is a likelihood ratio that expresses the odds that the genetic data would be observed if the parent -offspring pair tested is true than if the pair is really from a random unrelated person. The probability of exclusion for single parent statistics gives the proportion of the given population that does not contain the types to be a biological parent of the child based on the child's DNA profile.

Remember, the probability of exclusion provided with Popstats in single parentage statistics is always for the sample from the child (entered as the Reference); therefore, this impacts the report conclusion wording.

24.1.5 Other Calculations for Kinship such as Full and Half Sibship

Additional relatedness statistics can be generated using Popstats. DNA profiles entered as Forensic Single Source with Relatedness Reports generated calculate the probability of person with a given degree of relatedness having the identical DNA profile as the profile observed in casework. Kinship likelihood ratios can also be entered for a range of possible relatedness for the sources of DNA. Based on the DNA profiles entered, the resulting ratios provide statistics for the selected kinship relationship between two individuals.

24.1.6 Scale of Verbal Qualifiers for Likelihood Ratios – *include in kinship results*

Likelihood Ratio	Verbal Qualifier
1	Uninformative
2-99	Limited Support
100-9,999	Moderate Support
10,000-999,999	Strong Support
≥1,000,000	Very Strong Support

24.2 References

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25 REPORT WORDING GUIDELINES

The following is a guideline intended to help achieve uniform content and style in technical reports. Departures from the statements below will at times be necessary to accommodate specific circumstances. Reporting that is highly accurate, grammatically correct, and easily understood by non-scientific readers shall be acceptable.

A supplemental report is generated in LIMS.Net by selecting the previously issued report and clicking on the "Supplemental" button on the Reports tab. This report draft will include the previously issued report content in addition to the new findings and conclusions.

General practice to follow for taking additional samples for DNA testing and supplemental reports in LIMS.Net:

- If additional FB (screening procedure such as AP) is performed on an item after the initial report. If additional testing will be performed with a different STR kit on a DNA extract from a sample.
- If you take an additional sample from an item for DNA testing (no additional FB testing and even if from the same area, e.g. swab vs cutting or re-swab).

25.1 Discrepancies

Situation	Action
Item is different than stated on submission sheet – BCI error	~~Contact ER~~ – correct submission sheet and mail new submission sheet to department. Use corrected information in report.
Item different than stated on sub sheet and/or packaging	Leave item description as submitted followed by: **found to contain/be [actual item found] OR**

	found to contain ___ labeled ___
Item labeled differently than on submission sheet	Leave item description as submitted followed by: **found to be labeled [how labeled on packaging]**
Item quantity: description specifically states a # of swabs different than the # found	Leave item description as submitted followed by: **found to contain [actual quantity found]**
Item contains evidence stated on sub sheet along with other items	Leave item description as submitted followed by: - **also found to contain** [list only the additional items found]
Standards – not labeled with name on any packaging	Leave item description as submitted followed by: - **found to contain unlabeled swabs** - Subsequent reports will refer to them as unlabeled swabs - In Remarks, request new standard ("Please submit…")
Standards – labeled on inner and/or outer packaging	OK as is. **Does not** include standards that are labeled: "Suspect – John Doe, Victim – Jane Doe" with no clarification on packaging as to which one the sample originated from – these are considered unlabeled
	If discrepancy in the name itself use: - **found to be labeled…**
Do NOT discrepancy	- Minor spelling differences – just correct the error - Swab vs. swabs (no specific quantity specified at submission) – just add/remove the "s" as needed - Different # of reference standard swabs (remove the reference to the number of swabs from the Item description)

25.2 FB Report Wording Guidelines

The following document is a guideline intended to help achieve uniform content and style in technical reports. Departures from the statements below will at times be necessary to accommodate specific circumstances. Reporting that is highly accurate, grammatically correct, and easily understood by non-scientific readers shall be acceptable.

FB body fluid testing results table statements	
Result is…	**Conclusion**
ALS- /(visual exam neg for bld)	No stains for analysis
ALS- /(visual exam pos for bld)	No stains for semen analysis or No additional stains for analysis
AP +	Positive for acid phosphatase activity*
AP-	Negative for acid phosphatase activity*
~~* AP Footnote~~	~~*Acid phosphatase is found in semen, saliva, vaginal secretions, and some bacteria~~
Sperm Search+	Semen identified
AP +, Sperm Search -	Positive for acid phosphatase activity*, no sperm identified

Sperm Search -	No semen identified
Sperm Search + (one cell)	Single sperm cell identified
Sperm Search + (few) – typically for testing of non-orifice swab evidence	Trace amounts of semen identified
Sperm Search + (few) - orifice swab	Semen identified
Sperm Search -/PSA+	Presumptive positive for seminal fluid, no sperm identified
Sperm Search -/PSA-	No semen identified
Sperm Search QNC	Inconclusive for semen due to cell(s) lacking definitive morphological sperm cell characteristics
Sperm Search QNC/PSA-	Inconclusive for semen due to cell(s) lacking definitive morphological sperm cell characteristics
Sperm Search QNC/PSA+	Presumptive positive for seminal fluid, inconclusive for semen due to cell(s) lacking definitive morphological sperm cell characteristics
Sperm Search -/PSA unable to document	No semen identified
W/L+	Amylase identified
W/L-	No amylase identified
W/L unable to document	No amylase identified
TMB +	Presumptive positive for blood
TMB -	No blood identified
TMB inconclusive	Inconclusive for blood due to insufficient information obtained from the testing
HemDirect +	Human blood identified
HemDirect -	No human blood identified
Creatinine +	Creatinine identified
Creatinine -	No creatinine identified
Urobilinogen +	Urobilinogen identified
Urobilinogen -	No urobilinogen identified
Inmate case, no circled area	No circled area for analysis
Inmate case with unknown fluid thrown; creatinine negative, amylase test results in speckling only	No creatinine identified Amylase identified
DNA standard retained	Sample collected for DNA analysis
Item swabbed/retained, no testing	Sample collected for DNA analysis
Item not examined	Not examined
Very moldy/extremely dirty	Not suitable for analysis due to condition of item

Hair statements for FB results table
Note: Mito referrals for violent cases only. Discuss individual case circumstances with DNA management prior to writing report. Include report wording for body fluids as needed.

Description	Conclusion
Pubic hair/Head hair standard	No stains for analysis Found to be empty Not examined
Pubic hair combings	Trace debris present No trace debris present No stains for analysis Not examined
Trace debris from Item X	Not examined Suitable for nuclear DNA analysis Not suitable for nuclear DNA analysis Not suitable for nuclear DNA analysis; may be suitable for mitochondrial DNA analysis

25.3 DNA Report Wording Guidelines

Situations	DNA Conclusions
Single source	DNA profile consistent with suspect/victim – The estimated frequency of occurrence of the DNA profile is 1 in X^ unrelated individuals. Suspect/Victim – excluded Unknown [male/female] – sufficient for comparison
Mixture –no major Non-differential	Mixture (X contributors) Consistent with [contributions from]: • Victim – expected contributor • Suspect – The estimated frequency of occurrence of the DNA profile [not attributed to (victim)] is 1 in X^ unrelated individuals. • Suspect – The estimated proportion of the population that cannot be excluded as possible contributors to the mixture of DNA profiles is 1 in X^ unrelated individuals. • Unknown [male/female/includes male] – sufficient for comparison Suspect/Victim – inconclusive [due to insufficient data/due to relatedness/due to the number of contributors] Suspect/Victim – excluded
Mixture – major single source or clear major mixture Non-differential	Mixture (X major contributors) Major - Consistent with [contributions from]: • Victim – expected contributor

Reference to a male portion should generally be used when it is not already apparent that male DNA is present.	• Suspect – The estimated frequency of occurrence of the [major] DNA profile [not attributed to (victim)] is 1 in X^ unrelated individuals. • Suspect – The estimated proportion of the population that cannot be excluded as possible major contributors to the mixture of DNA profiles is 1 in X^ unrelated individuals. • Unknown [male/female/includes male] – sufficient for comparison Suspect/Victim – inconclusive [due to insufficient data/due to relatedness/due to the number of contributors] If one major Suspect – not the major contributor Suspect, Victim – not the major contributor If multiple majors Suspect – not a major contributor Suspect, Victim – not major contributors Suspect/Victim – excluded The remainder of this mixture contains DNA [, at least some portion of which is from a male,] that is not of sufficient quality for comparison to a standard from any individual.
Differential Major section identifier may be used for clarity. For a two person mixture with carryover between fractions, do not add “major” or “minor” for the single source stat to the report to avoid possible interpretation by customer as sample having a third contributor. Reference to a male portion should generally be used when it is not already apparent that male DNA is present.	Mixture (X [major] contributors) [Major -] Consistent with [contributions from]: • Victim – expected contributor • Suspect – The estimated frequency of occurrence of the [major] DNA profile [in the sperm/non-sperm fraction, not attributed to (name)] is 1 in X^ unrelated individuals. • Suspect – The estimated proportion of the population that cannot be excluded as possible major contributors to the mixture of DNA profiles in the sperm/non-sperm fraction is 1 in X^ unrelated individuals. • Unknown [male/female/includes male] – sufficient for comparison Suspect/Victim – inconclusive [due to insufficient data/due to relatedness/due to the number of contributors] If one major Suspect – not the major contributor Suspect, Victim – not the major contributor

	If multiple majors Suspect – not a major contributor Suspect, Victim – not major contributors Suspect/Victim – excluded The remainder of this mixture contains DNA [,at least some portion of which is from a male,] that is not of sufficient quality for comparison to a standard from any individual.
Differential/Non-differential – matches victim on intimate item when looking for foreign DNA Do not use if the victim's DNA answers the question of the source of the stain (e.g., non-menstrual blood stain on item for which the source of the stain is the question).	No DNA profile foreign to [Victim]
Profile could only be used to exclude. Reference to a male portion should generally be used in sexual assault samples only.	The DNA profile [,at least some portion of which is from a male,] is not sufficient for inclusion. Mixture Consistent with contributions from two individuals: • Victim – expected contributor The remainder of this mixture contains DNA from a male that is not sufficient for inclusion. Suspect/Victim – excluded Suspect/Victim – inconclusive due to insufficient data/due to relatedness
DNA Standard	Profile used for comparison purposes Not suitable for use as a reference standard
Complex mixture (includes unresolvable 4 person mixtures) / Partial profile cannot include or exclude "…male…" should generally be used in sexual assault samples only.	The DNA profile [,at least some portion of which is from a male,] is not of sufficient quality for comparison [due to insufficient data/due to the number of contributors]. The DNA profile [,at least some portion of which is from a male,] is not of sufficient quality for comparison due to insufficient data and the number of potential contributors.
Limited additional minor peaks consistent with victim	Additional data consistent with [victim] or # additional peaks consistent with [victim]
No DNA profile	No DNA profile
Item not examined	Not examined
Item swabbed/retained, no testing	No DNA analysis
When item combined	See Item X

No Y STR testing based on quant and report needed	No Y-STR testing performed due to insufficient male DNA
5 or more additional peaks that are not in forward or reverse stutter positions. Consider peaks above the analytical threshold and those below distinct from baseline. No statement is needed if less than 5 additional peaks above or below threshold.	The remainder of this mixture contains DNA that is not of sufficient quality for comparison to a standard from any individual.
Sample with control failure	DNA results inconclusive, see remarks

Footnotes	
* AP Footnote	*Acid phosphatase is found in semen, saliva, vaginal secretions, and some bacteria
Database used for frequency calculations	^ Based on the national database provided by the National Institute of Standards and Technology
For use as needed in cases in which a blind swab type sample is taken and cohabitation/caregiver should be considered.	#The occurrence of incidental transfer of DNA between household members is well established. The circumstances surrounding the deposition of the male/caregiver/family member DNA herein cannot be determined based on DNA results.
When the ^{Y} symbol is used next to a sample conclusion to denote Y -STR testing eligibility	^{Y} Sample eligible for Y-STR analysis
Changed interpretation	@ Based on current interpretation guidelines
Now using ArmedXpert for stat	** Re-calculated using ArmedXpert software

Kinship Report Wording

Kinship Conclusions	
Situation	**Conclusions**
Suspect included – Standard trio	[Suspect] cannot be excluded as the biological father of [child/fetus]. Based on the national database provided by the National Institute of Standards and Technology, the evidence is XXX times more likely if [suspect] is the biological father of [child/fetus] than if a random man is the biological father of [child/fetus].

	Using a prior probability of 0.5, the probability that [suspect] is the biological father of [child/fetus] is XX.XXXX% (use 4 decimal places)
Reverse Parentage	[Child/fetus/donor] cannot be excluded as being the biological child of [father] and [mother]. Based on the national database provided by the National Institute of Standards and Technology, the evidence is XXX times more likely if [Child/fetus/donor] is a biological child of [father] and [mother] than if [Child/fetus/donor] is a biological child of two randomly selected individuals. Given the genetic profiles of [mother] and [father], the probability of excluding a random individual as being the biological offspring is XX.XXXX% (use 4 decimal places).
Suspect included – Single parentage (Using Popstats, the RMNE is based on the profile entered as the reference which is generally for the child)	[Suspect/known] cannot be excluded as being the biological [father/mother] of [child/fetus/donor]. Based on the national database provided by the National Institute of Standards and Technology, the evidence is XXX times more likely if [suspect/known] is the biological [father/mother] of [child/fetus/donor] than if a random individual is the biological [father/mother] of [child/fetus/donor]. Given the genetic profile of the [child/fetus/donor-entered as the Reference in Popstats], the probability of excluding a random [male/female] as the biological [father/mother] is XX.XXXX% (use 4 decimal places). **Note the RMNE should be based on the child's profile for this conclusion.
Relatedness for a single source profile	The probability of a [full sib, half sib, etc.] having the same DNA profile as [suspect] is approximately 1 in XXX.
Relatedness of individuals	Based on the national database provided by the National Institute of Standards and Technology, the evidence is XXX times more likely if [person X] and [person Y] are [full sib, half sib, etc.] than if random unrelated individuals.
Suspect excluded	[Suspect] is not the biological father of [child/fetus].
Standard run in new kit in case A to compare to case B. This is to report new kit testing for standard in case A	Additional DNA testing performed on this item. Profile used for comparison in a separate case. Please contact this office for additional information.

25.4 Remarks

Disposition of items	
Situation	**Remarks**
FB only report retain samples	All samples will be retained by the laboratory until DNA testing is completed.

Positive sperm search slides made	Prepared microscope slides from Item X with semen identified were retained and will be returned to the submitting agency.
Trace debris not examined	The trace debris from Item X was not examined.
NIBIN retained sample Remarks section	All evidence items are being returned to the submitting agency. If a correlation is made in NIBIN on firearms related evidence, please contact DNA laboratory management for possible testing.
Weapons Under Disability case with no standard– firearm swabs to be returned	All evidence items are being returned to the submitting agency. Upon submission of a DNA reference standard consisting of two oral swabs from the suspect, please resubmit Container XXX.
FB only report do not retain samples for DNA:	All evidence will be returned to the submitting agency.
Container(s) to be returned - we cannot proceed without consumption authorization	All evidence items are being returned to the submitting agency. Upon submission of consumption authorization, please resubmit Containers 1 and 2.
Sample consumed	Item X was consumed during analysis. Additional sample from the other items is available should independent analysis be requested. All remaining items will be returned to the submitting agency. The remaining DNA extracts will be retained by the laboratory. (this is only used when an item cannot be re-swabbed/re-cut and is consumed)
Sample not consumed	Additional sample from each item is available should independent analysis be requested. All remaining items will be returned to the submitting agency. The remaining DNA extracts will be retained by the laboratory.
Extract consumed	Additional sample from each item is available should independent analysis be requested. All remaining items will be returned to the submitting agency. The DNA extract for Item X was consumed during analysis. The remaining DNA extracts will be retained by the laboratory.
Swab collected in different lab than where tested	The swab of X (Item Y) was collected in the Bowling Green/London/Richfield laboratory. The remainder of the testing was performed in the Richfield/London laboratory.
Extract transferred from lab without a report being issued in the extracting lab	The DNA extracts from Items X, Y, and Z were transported to the London Laboratory for Y-STR testing. The data were analyzed and reported in the Richfield laboratory.
FB Amylase testing – mist/spatter pattern	The positive amylase area is similar to a mist or spatter pattern. Coughing and sneezing cannot be ruled out as possible origins of the stain.

If case will go to DNA even if we don't get a standard and FB report only report issued	Please submit a reference [elimination] standard consisting of two oral swabs from the suspect [consensual partner]. Or Please submit reference standards consisting of two oral swabs each from the consensual partner and any suspects.
Need consumption authorization or unknown if capital case	It is our opinion that the DNA analysis most likely to produce a DNA profile from Item X requires consuming the entire sample. Written authorization from the prosecutor may be required before testing can begin on this sample. Please contact this office for additional information. Samples are not being forwarded for DNA analysis at this time.
Need standard for Ys and no other DNA testing will be performed	It is our opinion that the DNA analysis procedure most likely to produce data foreign to (victim) is Y-STR analysis. Submission of a reference standard consisting of two oral swabs from (suspect) and resubmission of container(s) xxx are required before DNA analysis will begin. Samples are not being forwarded for DNA analysis at this time.
All items are negative	Additional items of evidence may be submitted. Samples are not being forwarded for DNA analysis at this time.
No suspect, hair without root, case is one we might send forward for mito if a suspect is developed	Not suitable for nuclear DNA analysis (in Remarks, add: The hair from Item X may be suitable for mitochondrial DNA analysis. If a suspect is developed, please resubmit the hair along with a reference standard consisting of two oral swabs.)
Consent partner status unknown but profile will be entered	Please ensure any available elimination standards are submitted.
Item not examined – will usually be in the table	Item x was not examined.
Additional DNA testing to be completed	Additional analysis will be performed on Item XXX/Items XXX.
Have profile and standards, have unidentified probative profile	(Additional) DNA comparisons can be made upon submission of a reference standard consisting of two oral swabs from (a suspect, the consensual partner, the victim).
Sending samples for Y-STR	Samples are being forwarded for Y-STR analysis which may be helpful in determining the source of any male DNA that is present.
May send samples for Y-STR	Y-STR analysis may be helpful in determining the source of any male DNA that is present. Please contact this office for additional information.

	Should a reference standard consisting of two oral swabs from (suspect) be submitted, samples can be forwarded for Y-STR analysis. Please ensure any available elimination standards are submitted. Should a suspect be developed and a reference standard consisting of two oral swabs be submitted, samples can be forwarded for Y-STR analysis. Please ensure any available elimination standards are submitted. Y-STR analysis may be helpful in determining the source of any male DNA that is present. Submission of a reference standard consisting of two oral swabs from (suspect) [and resubmission of container(s) X] is/are required before DNA analysis will proceed.
Have profile, entered into CODIS – applies to both CODIS and suspect case samples. Y-STR profiles are not entered into CODIS. No-suspect paternity, paternal haplotype entered into CODIS	Eligible DNA profiles (Items X, Y, and Z) have been entered into the CODIS database in accordance with state and national regulations, where regular searches will be performed. If investigative information becomes available or a profile is removed from CODIS, your agency will be notified.
Have profile, not entered (no suspect standard and the evidence profile not eligible)	The forensic DNA profile developed was not suitable for CODIS entry.
Reporting a profile removed from CODIS in a subsequent report	The forensic DNA profile (Item X) has been removed from the CODIS database.
One time CODIS search	A one-time search of the (local/state) CODIS database was performed on all eligible DNA profiles. No investigative information was obtained. **OR** Your agency will be notified of any available investigative information.
Need more info for CODIS eligibility	Based on the information provided regarding Item XXX, any forensic DNA profiles developed are not eligible for CODIS entry. If information becomes available to indicate a crime likely occurred, please contact this office.
Need standard for CODIS	The forensic DNA profiles developed were not eligible for CODIS entry without a reference standard from XXX.
Replacing suspect standard with evidence	Eligible DNA profiles (Items X, Y replacing W, and Z) have been entered into the CODIS database in accordance with state and national regulations, where regular searches will be performed. If investigative information becomes available

	or a profile is removed from CODIS, your agency will be notified.
Cohabitation potential an issue	The occurrence of incidental transfer of DNA between household members is well established. The circumstances surrounding the deposition of the male DNA herein cannot be determined based on DNA results.
CODIS case, have profile, need standard	Should a suspect be developed, please submit a reference standard consisting of two oral swabs.
Amended report for error	This report replaces the original report by NAME issued DATE in its entirety.
Additional work *(std submitted, reinterpret profile using current interpretation guidelines, now DNSFC*) on the same extract or FB testing of item	This report supplements the original testing performed on Item(s) X issued in the report issued DATE.
Original analyst cannot testify	This report was re-interpreted due to the lack of availability for testimony purposes of the original reporting Forensic Scientist NAME originally issued DATE.
QA reanalysis – same findings	The above listed items were re-examined for quality assurance purposes. The findings concur with those outlined in the report issued by XX, dated DATE.
QA reanalysis – different findings	Varies - consult DNA management
Control failure remark – evidence available to re-test	Data from an initial analysis of Item(s) X was not used due to a control failure. Additional analysis was performed and reported above.
Control failure remark – evidence consumed and not available for re-testing	Data from the analysis of Item(s) X was not used due to a control failure.
This or similar can be used when kit manufacturing or collection profile is obtained.	*Contains extraneous DNA profile known to be associated with packaging material contained within sexual assault kits*
Original analyst not available	*The data were re-interpreted and reported due to the lack of availability for testimony purposes of the original reporting Forensic Scientist NAME originally issued DATE.*

25.5 Analytical Detail

Analytical Detail section	
A description of the methods used for testing should be included in the Analytical Detail section of the report. Select the appropriate Method Statements based on the testing performed. The combined statements should summarize the methods used for all samples.	
Situation	**Analytical Detail**
ALS/IR Camera	An [alternate light source/infrared camera] was used to assist in detection of stains.

Semen (AP/PSA/Sperm Search)	Presumptive analysis for semen was performed using chemical testing. [Presumptive analysis for seminal fluid, the liquid component of semen, was performed using immunological testing.] [Microscopic analysis was used for confirmation of semen.]
Blood (TMB/HemDirect)	Presumptive analysis for blood was performed using chemical testing. [Confirmation of human blood was performed using immunological testing which may cross-react with higher primate and ferret blood.]
Wurster/Laux	Analysis for amylase, a component of saliva and other body fluids, was performed using a chemical test.
Creatinine	Analysis for creatinine, a component of urine and other body fluids, was performed using a chemical test.
Urobilinogen	Analysis for urobilinogen, a component of feces and other body fluids, was performed using a chemical test.
Trace debris (examined)	Microscopic examination of trace debris was performed to determine if a hair root is present for nuclear DNA analysis.
Results: Allele Table in report	DNA profiling was performed using PCR with the Globalfiler® STR kit. The results are listed in Table 1.
Results: No Allele Table in report	DNA profiling was performed using PCR with the Globalfiler® STR kit on samples from Item x. (Only refer to the parent item not subs – unless needed for clarity)
Results: Referring to some previously reported results – same case. Clarify which samples are newly tested.	DNA profiling was performed using PCR with the Globalfiler® STR kit on samples from Item x. The additional samples listed above were previously analyzed. See report(s) issued X (and Y).
Results: Referring to previously reported results – different case	DNA profiling was performed using PCR with the Globalfiler® STR kit on samples from Item x and compared to a previously analyzed sample from BCI 08-99999 Item y (see report issued xxx). Only use “see report issued” if referencing report to same agency.
Results: Referring to previously reported vendor data from same case Under the submitted items reference the vendor lab sample name as	DNA profiling was performed using PCR with the Globalfiler® STR kit on samples from Item x and compared to a previously analyzed sample from Item y (see [vendor name] case [vendor case #] report dated xxx). This sample is also referred to as…
Results: Referring to samples in new report for which no new testing is-reported	DNA profiling previously performed on a sample from Item x (see report issued xxx) was compared to a previously analyzed sample from BCI 08-99999 Item y (see report issued xxx). – Only use “see report issued” if referencing report to same agency

Analysis halted to send for Ys	DNA analysis was begun on the vaginal swabs (Item X) and anal swabs (Item y) but was halted due to the limited quantity of male DNA detected. (Remarks would state: Samples from the vaginal swabs and anal swabs, along with the standard from Suspect (Item Z) are being forwarded for Y-STR analysis.) ** may also need to add consumption wording if needed
Analysis halted	DNA analysis was begun on Item X and halted at the request of [Det. Or Pros. Name].
Standard re-tested with Globalfiler	DNA profiling was performed using PCR with the Globalfiler® STR kit on a sample from Item X to be used for future comparison purposes as needed.

25.6 Other Guidelines

1. If there is an event during the testing process that requires an additional sample to be taken, then a statement is required in the Remarks section of the report. Issues with controls, robots, or other instrumentation may result in the need for use of this statement. If insufficient results at quant or in the STR profile require additional testing and sample use, this does not require a statement. Use only when additional sample must be used due to a negative event in the testing process. Successful reamp of a sample (failed initially for positive/neg issue or something during amp set-up) would not trigger a statement since no additional sample was required. This only applies to evidence samples, not reference standards.

   Conclusion and remark options are listed in the tables above for reference and available in the report wording matrix in LIMS.net.

2. It is the responsibility of the writer and technical reviewer to evaluate the overall case results. Additional testing may be indicated. Considerations include:
   - Would an additional sample or reamplification of an item potentially yield more complete results?
   - Are the DNA results consistent with the screening results (e.g., a strong male profile in the sperm fraction and a positive sperm search result)? Because DNA testing is more sensitive than screening methods, partial/weak DNA results may have low or negative screening test results;
   - Should a confirmatory test for blood be performed if strong TMB and negative DNA results?

3. When subsequent DNA reports are issued for cases where additional testing was performed, the report will be inclusive of the previously reported DNA results/conclusions for all samples that

had profiles sufficient for comparison. This practice will help ensure conclusions for all reference standards are reported and available for court testimony, if needed.

The following are additional guidelines:

- It is optional to make the report completely inclusive by including FB results, prior reporting for samples with no DNA profiles foreign to the DNA profile of a reference standard, and/or samples with no/insufficient DNA profiles for comparison.
- Comparison to newly submitted reference standards will be made to new and previously reported samples that have profiles sufficient for comparison even when the source of the DNA is explained by submitted reference standards. This includes comparison of the Globalfiler profile for reference standards submitted for Y-filer Plus testing.
    - One exception is for findings of no DNA profile foreign to the expected contributor – this does not require additional exclusion statements to be made or carried through to subsequent reports.
- Exceptions may be made for cases in which many rounds of testing have been performed. Consult with DNA lab management in these cases.

4. For reporting the presence of male DNA, at least two of the following should be detected: a Y quant, a callable Y-allele at Amelogenin, indel, or DYS391.
5. If an Allele Table is included with the Report, list evidence samples with alleles in numerical order as follows:
    1. Evidence (differentials) in numerical order
    2. Evidence (non-differentials) in numerical order
    3. Standards
6. For reference in reports to unknown single source or major profiles, use the following nomenclature.
   One unknown profile: “Unknown Male/Female”
   Additional occurrence of the same profile without needing to identify another unknown profile: “Same unknown male/female”
   Multiple unknown profiles: “Unknown Male/Female 1” and “Unknown Male/Female 2”, etc.
   If a standard is submitted for an unknown profile, replace the identifier with the name of the individual. Maintain the original numbering for unknown profiles. Do not modify the numbering.
7. When called to another section to evaluate an item, add the following wording to the item description in the submitted section of the report: Based on consultation with the [Latent Prints] section, subsequent DNA examination was performed.
8. Trash pull samples:

Below is an example to guide reporting for trash pull sample submissions with LIMS.net. No statistic frequencies will be included for evidence profiles consistent with profiles from trash pull items, note the # footnote wording. More reliable secondary standards may be handled differently.

The trash pull comparison reports only need to be the relevant unknown profiles. A comprehensive report is not required.

Item	DNA Conclusions
Table 1	
1.8.2 Skin stain swab – "Chest"	Positive for acid phosphatase activity* Mixture (2 major contributors) Major: • Unknown male – consistent with male profile G obtained from Item 6.3# • Victim – expected contributor Mario – not a major contributor The remainder of this mixture contains DNA that is not of sufficient quality for comparison to a standard from any individual.
1.10.1 DNA standard – Victim	Profile used for comparison purposes
2 DNA standard – Mario	Profile used for comparison purposes
Table 2 Comparison samples submitted from Trash Pull(s)	
3.1 Cigarette butt	Male profile A used for comparison purposes
4.1 Cigarette butt	Male profile B used for comparison purposes
5.1 Swab from mouth/cap of bottle	Male profile C used for comparison purposes
5.5 Swab from mouth of can	Major male profile C used for comparison purposes
5.2 Swab from mouth/cap of bottle	Female profile D used for comparison purposes
5.3 Swab from mouth of bottle	Female profile E used for comparison purposes
5.4 Swab from mouth of can	The DNA profile is not of sufficient quality for comparison due to insufficient data.
6.1 Swab from can	Female profile F used for comparison purposes
6.2 Swab from can	Major female profile F used for comparison purposes
6.3 Swab from mouth/cap of bottle	Male profile G used for comparison purposes

*Acid phosphatase is found in semen, saliva, vaginal secretions, and some bacteria
DNA conclusions with statistical analysis can be made upon submission of a reference standard consisting of two oral swabs from the donor of the DNA from Item 6.3.

Remarks
Unless stated in Table 1, profiles in Table 2 are not consistent with the Unknown profile(s) in Table 1.

Single source and major single source profiles obtained from trash pull samples are referenced in Table 2 and were used for comparison purposes. Any minor data is not of sufficient quality for comparison.

26 Y-FILER PLUS STR PROFILING

26.1 Introduction

The AmpFLSTR Y-filer Plus® kit contains primers to amplify the short tandem repeat loci DYS576, DYS389I, DYS365, DYS389II, DYS627, DYS460, DYS458, DYS19, YGATAH4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS518, DYS570, DYS437, DYS385, DYS449, DYS393, DYS439, DYS481, DYFS387S1, and DYS533.
Storage of kit components: Store unopened kits frozen. For the single in-use kit, store components refrigerated. Ladders go to the PCR area.

NOTE: Y-filer Plus STR testing is performed based on case information and DNA quantitation results. If the Quantifiler Trio results indicate an amplification target of < 0.02 ng male DNA from orifice/skin stain swabs or < 0.125 ng for other sample types (e.g. clothing), no Y-filer Plus testing needs to be performed. This specialized male specific testing is reserved for eligible cases with considerations of caregiver, cohabitation, sample type, and other relevant case facts.

Additional guidance
If the Quantifiler Trio results indicate an amplification target of ≥0.02 ng male DNA from the samples types listed below, these can be sent forward for Y-STR testing:

- Orifice samples (vaginal, anal, perianal, oral)
- Skin swabs from covered areas on the body (genitals, breasts, sites of ejaculation such as back or abdomen)

If the Quantifiler Trio results indicate an amplification target of ≥0.125 ng male DNA from other sample types listed below, these can be sent forward for Y-STR testing:

- Clothing including underwear
- Drug baggies
- Skin swabs from uncovered areas of the body based in unique case circumstances
- Neck swabs
- Circumoral swabs collected up to 24 hours post incident

Case scenarios should be considered along with STR and Quantifiler Trio results when selecting and sending samples forward for Y-STR testing. Consult with a supervisor for case specific scenarios and consideration of Y-STR testing.

26.2 PCR Amplification

1. Prepare DNA samples to a target of 1.0 ng male DNA in a volume of 10 µl.
2. Amplification controls consist of positive control DNA from the Yfiler Plus kit and 10 µl of the same TE used to set up the reactions.

3. Label the controls:
   Pos_amp date-initials Example: **Pos_100205EB**
   Neg-amp date-initials Example: **Neg010516EB**
4. Prepare a Yfiler Plus Amplification master mix. Thaw (if necessary) the Yfiler Plus Master Mix and Primers Tubes. Briefly vortex and spin the tubes. Make an amplification master mix consisting of 10 µl master mix and 5 µl primers per sample.
5. Dispense 15 µl of the mixture into each sample well.
6. Add 10 µl sample to each tube.
7. Amplification Parameters for the Proflex Thermal Cycler (about 1.5 hours):

Parameter	°C/min
Hold	95/1 min
Denature	94/4 sec
Anneal/Extend	61.5/1 min
Cycles	30
Final Extension	60/22 min
Hold	4/∞

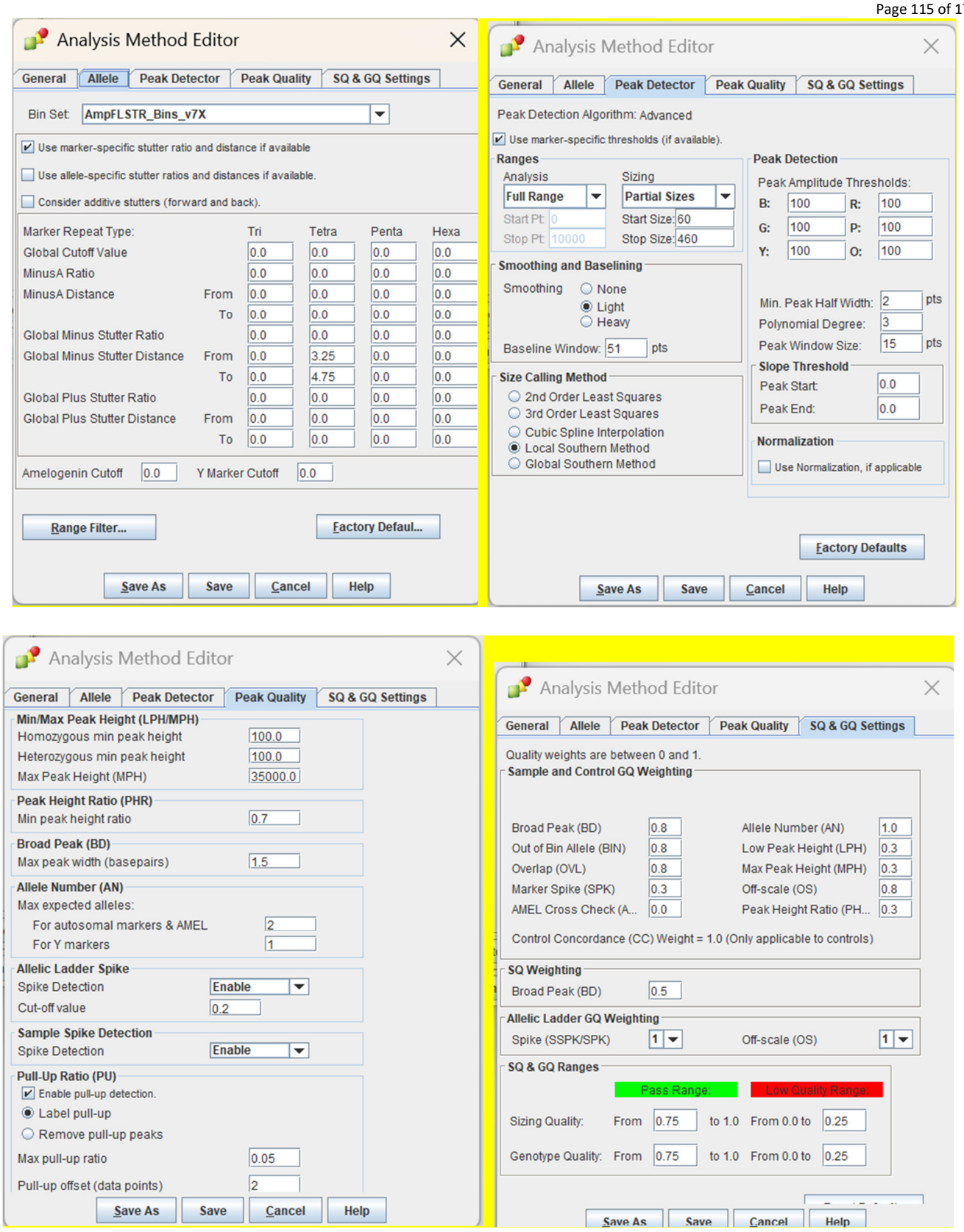
26.3 Capillary Electrophoresis

1. Follow Globalfiler set-up directions. Prepare CE master mix consisting of 0.4 µl LIZ internal lane standard and 9.6 µl formamide. Add 1.0 µL Y-STR amp product to 10 µl CE master mix. Run one allelic ladder per 24 samples. Use 1.0 µl allelic ladder stock.
2. Use 24-second injection.

26.4 Analyzing Y-Filer Plus

26.4.1 Genemapper IDX v1.7 Software

1. *Follow project creation procedure as for Globalfiler.*
2. *Y-filer Plus settings:*
   Analysis Method = Y+ Casework 02052025
   Panel = Yfiler_Plus_Panel_v4.2X
   Bin = AmpFLSTR_Bins_v7X
   Stutter ratio = marker specific (values must be loaded into the Panel Manager)

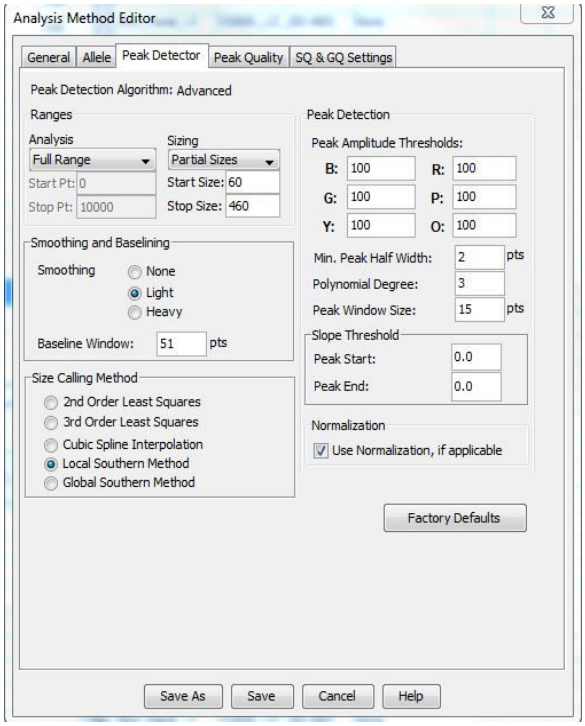


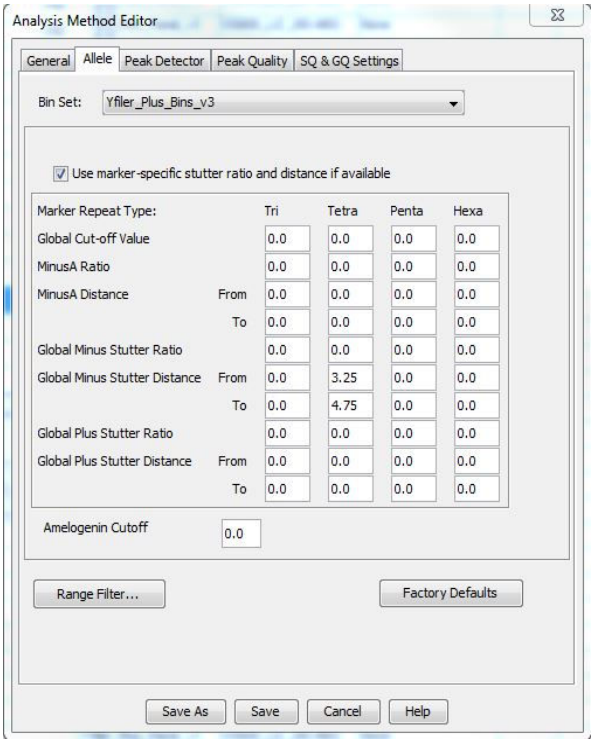
26.4.2 *Genemapper IDX v1.4 software*

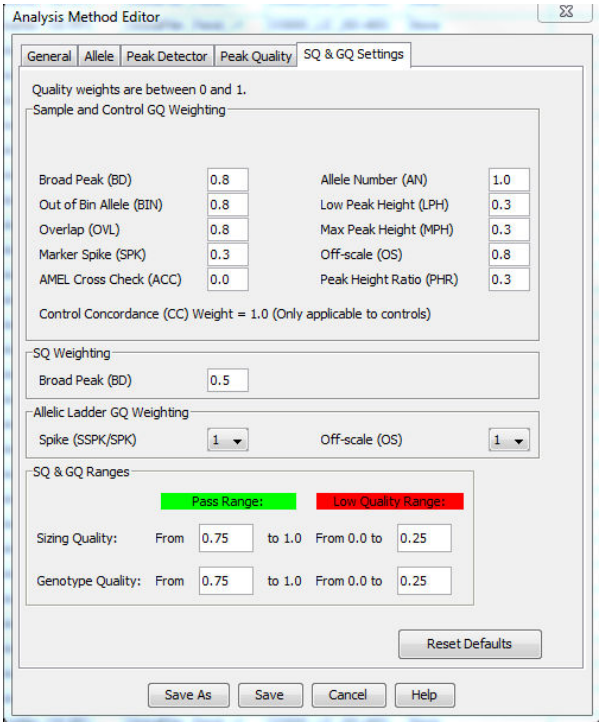
1. Follow project creation procedure as for Globalfiler.
2. Y-filer Plus settings:

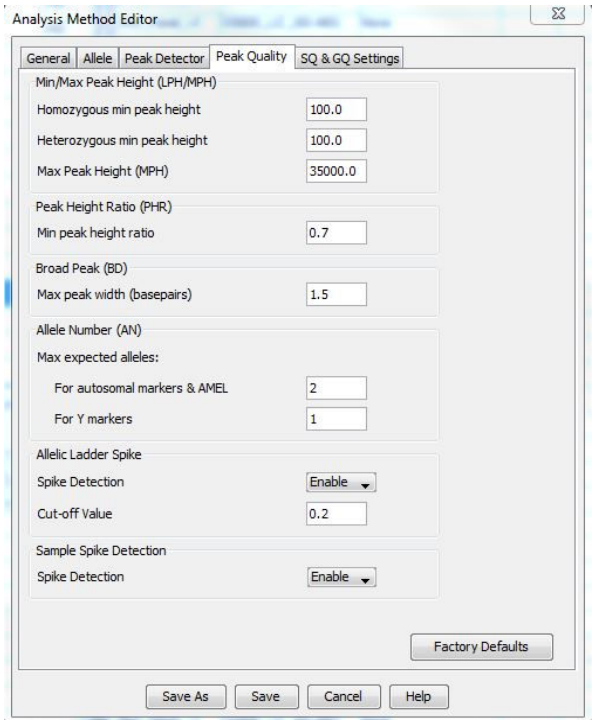
   Analysis Method = Yfiler Plus

Panel = Yfiler_Plus_Panel_v3
Bin = Yfiler_Plus_Bins_v3
Stutter ratio = marker specific (values must be loaded into the Panel Manager)









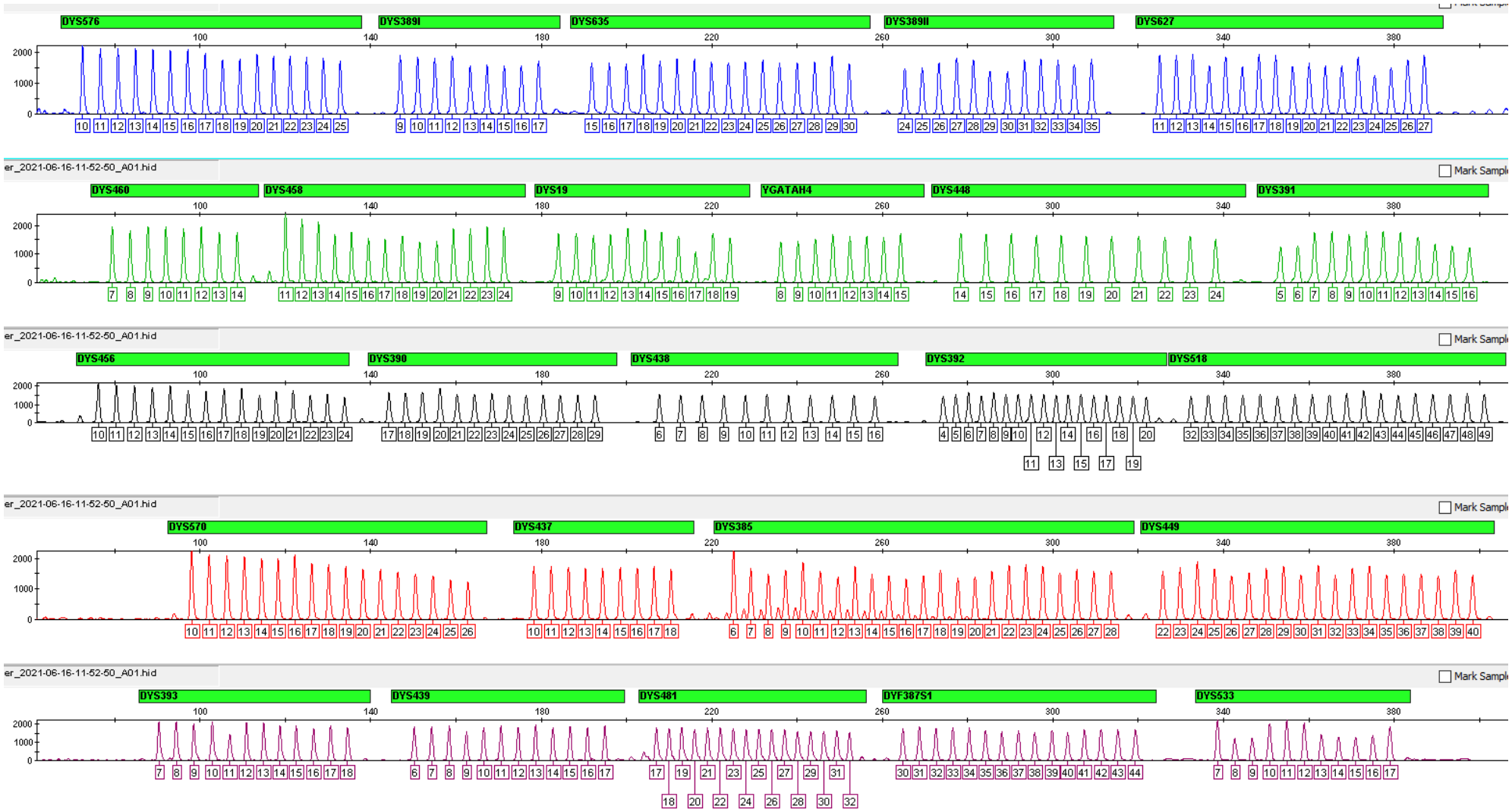
25.4.3 Additional Analysis

GM IDX Yfiler Plus Stutter Filter Settings		
Locus	N - 4 (- 2)	N + 4 (+2)
DYS576	15.2	3.4
DYS389I	9.2	3.5
DYS635	13.4	3.3
DYS389II	18.8	3.7
DYS627	15.2 (2.7)	2.6
DYS460	11.7	4.3
DYS458	15.3	2.5
DYS19	12.7 (10.1)	3.7 (3.4)
YGATAH4	11.5	2.3
DYS448	4.7	2.3
DYS391	10.0	3.4
DYS456	15.4	3.7
DYS390	13.6	3.5
DYS438	5.9	2.8
DYS392	16.9	11.0
DYS518	25.5	4.9
DYS570	15.7	2.9
DYS437	8.1	1.7
DYS385	18.3	3.7
DYS449	23.2	4.2
DYS393	14.1	5.0
DYS439	9.9	3.4
DYS481	28.6 (9.6)	5.6
DYF387S1	15.7	0.0
DYS533	12 (1.9)	4.6

Y-Filer Plus Positive Control Alleles		
Locus	Kit 007	SMW
DYS576	19	16
DYS389I	13	12
DYS635	24	21
DYS389II	29	29
DYS627	21	19
DYS460	11	11
DYS458	17	16
DYS19	15	14
YGATAH4	13	11
DYS448	19	19
DYS391	11	10
DYS456	15	13
DYS390	24	24
DYS438	12	9
DYS392	13	11
DYS518	37	39
DYS570	17	17
DYS437	15	16
DYS385	11,14	13,17
DYS449	30	28
DYS393	13	12
DYS439	12	12
DYS481	22	24
DYF387S1	35,37	38
DYS533	13	12

1. Review raw data to troubleshoot sizing difficulties. Check Analysis Method Editor to verify that the sizing range (3000-6000) brackets the set of GS600 LIZ size standards as shown, all peaks are correctly labeled and no artifacts are labeled. The LIZ 600 internal size standard must have the expected size results for the 60.0, 80.0, 100.0, 114.0, 120.0, 140.0, 160.0, 180.0, 200.0, 214.0, 220.0, 240.0, 250.0, 260.0, 280.0, 300.0, 314.0, 320.0, 340.0, 360.0, 380.0, 400.0, 414.0, 420.0, 440.0, and 460.0 base pair fragments. The peaks must be sizable but do not necessarily have to be above 100 RFU.

2. To edit a particular size standard peak, right-click the peak.
3. Examine the allelic ladders to determine that the software has assigned all allele designations correctly and the peaks are at least 100 RFU. The following alleles at the specific loci must be labeled.


DYS576
DYS389I
DYS635
DYS389II
DYS627
DYS460
DYS458
DYS19
YGATAH4
DYS448
DYS391
DYS456
DYS390
DYS438
DYS392
DYS518
DYS570
DYS437
DYS385
DYS449
DYS393
DYS439
DYS481
DYF387S1
DYS533
Mark Sample

4. Verify that positive controls give the correct type. Rarely, if the positive control fails to amplify, a single-profile case sample can be re-run with a working positive control and then used as a secondary positive control.
5. Check for peaks in negative controls. If a non-artifact peak above threshold is detected in a manipulation blank or negative control, investigate following the Globalfiler procedure.
6. Review samples and mark artifacts: spikes, pull-up, raised base line, -A, dye blobs, and nonspecific female DNA amplification.
7. Print the e-grams.
    a. Base pair range for X-axis = 60-460
    b. Y-axis = scale to top of highest peak for each dye color
    c. Negative sample Y-axis = 75
    d. Print allele call and bp only
    e. Font size = 6
    f. Consider printing a second zoomed-in view where a very minor profile is present.

26.5 Interpretation

1. The analytical minimum and interpretational threshold is 100 RFU. The stochastic threshold is 400 RFU. Peaks above 30,000 RFU and their associated stutter should be interpreted with caution. True peaks (base broader than tip and somewhat symmetrical, and not dye blobs, spikes, crystals, or other artifacts) above the listed thresholds are callable peaks. For Y-filer Plus, the analytical threshold will be the same as the interpretational threshold (unless sample is a mixture).
2. Microvariants are named using the same syntax as Globalfiler.
3. Off-ladder alleles are named using >/< the largest or smallest ladder allele. If an allele is intermediate between two loci, assign it to the locus missing data. Check the bp size of OLs when making inclusions.
4. Genemapper ID-X stutter filtering is on, using the published ID-X locus-specific values. The stutter filter de-labels stutter peaks at or below the set values. Additional potential stutter may be marked manually. Actions of the stutter filter are not considered to be interpretation and may be overridden either way by the analyst.
5. A DNA profile may be from a single individual if one allele is observed at each locus, except DYS385a/b and DYF387S1 which may contain two alleles. The peak height ratios at duplicated loci are generally 50% or greater for single source samples down to ~.25 ng input. Allele sharing may impact this ratio.
6. Mixture criteria for normal template quantity amplifications
    a. Three or more loci contain more than one peak
    b. All potential artifacts are ruled out as causes of the peaks
    c. Based on chromosomal location, the extra peaks do not raise suspicion of duplication (JFS 50:853-859)
7. Where there is reasonable suspicion that the appearance of a mixture may result from one or more artifact, do not report a mixture. As a rule of thumb, do not report an additional contributor unless you would be able to make an inclusion given an appropriate reference standard. Rather, consider reporting additional peaks.
8. To the extent possible, review the unknown samples before the reference standards. Compare questioned profile to known profile. If the DNA profiles are distinctly different, the

individual is excluded. If the DNA profiles appear consistent, the individual cannot be excluded.

9. Known contributors (e.g. male victim, consensual partner) may be subtracted from mixtures.
10. Major-minor contributor profiles can be interpreted separately where the peak height of the major allele is 4X greater than the largest minor allele. If a small number of loci do not meet this requirement, exclude them from the statistical calculation. If more loci do not meet the 4X requirement, then the mixture is unresolvable.
11. Where degradation, drop-out or inhibition exists, at least 6 interpretable loci are required to make an inclusion. However, exclusions can be made with any number of loci.
12. Report a statistical frequency for all inclusions.

26.6 Statistical Calculations

1. Given the male lineage non-recombinatorial pattern of inheritance, haplotype frequencies are estimated using the counting method.
2. Due to the common duplication of the DYS385 and DYF387S1 loci, these loci should not be included in haplotype calculations if the zygosity (<300RFU) is questioned.
3. To calculate a single source frequency, go to http://yhrd.org/.
    a. From the "Search the Database" option, select to manually enter the haplotype then select the "Yfiler Plus" kit option and the "Y27" dataset to perform a masked search. After searching the haplotype, close the panel for the Worldwide option and add the "National Database (with Subpopulations, 2014 SWGDAM-compliant)" option. Print the YHRD report which will include the number of matches in the haplotype dataset and the 95% Upper Confidence Interval (UCI) for the United States (Overall).
    b. Keep the "Yfiler Plus" kit selected and change to the "Y17" dataset option to perform a transient search. After searching the haplotype, close the panel for the Worldwide option and add the "National Database (with Subpopulations, 2014 SWGDAM-compliant)" option. Print the YHRD report which will include the number of matches in the haplotype dataset and the 95% Upper Confidence Interval (UCI) for the United States (Overall).
4. Report the most discriminating search which is generally the one which gave the lowest proportion of matching haplotypes per number of profiles compared.
5. Frequency estimate calculations are made using the counting method. In cases where a Y STR profile is observed a particular number of times (X) in a database containing N profiles, its frequency (p) can be calculated using p = X/N
6. An upper bound confidence interval of 95% can be placed on the profile's frequency. In 95% of intervals constructed in this way, the true frequency is higher than this value; thus, with 95% confidence the actual haplotype frequency will be rarer than that provided by the estimate. The US Y-STR-suggested formula for calculating a 95% confidence limit is:

$$\sum_{k=0}^{x}\binom{n}{k}p_0^k(1-p_0)^{n-k}=0.05$$

Where: $\binom{n}{k}=\frac{n!}{k!(n-k)!}$ with n = successes in k trials

7. If no observations were made of the profile in the database, the following formula is used to estimate the frequency with a 95% confidence interval:

$$1 - (0.05)^{1/n} \qquad n = \text{database size}$$

8. If both autosomal and Y-STR data are collected on a sample, the product rule may be used to combine the autosomal STR genotype match probability and the Y-STR haplotype frequency information.
9. When reporting a statistic from the Y Chromosome Haplotype Database, include both the release number and date of the last update in the casework documentation. As the database is updated, statistical calculations may change.

26.7 Report Wording

Other report wording may follow the style used for Globalfiler.

Table Report

Situation	Y-STR DNA Conclusions
Partial profile or unresolvable mixtures	The Y-STR DNA profile is not of sufficient quality for comparison [due to insufficient data/due to the number of contributors].
No Y-STR DNA profile	No Y-STR DNA profile
Single source profile	Unknown male – sufficient for comparison
Single source Y profile with an inclusion	Y-STR DNA profile consistent with Suspect – Neither the listed individual nor any of his paternal male relatives can be eliminated as the [major] source of the Y-STR DNA profile [in the sperm fraction]. This Y-STR profile has been observed (X) times in the Y-Chromosome Haplotype Database of (Y) profiles and is not expected to occur more frequently than 1 in (Z) male individuals in the U.S. population[&].
Mixture with major Y profile with an inclusion, additional data not sufficient for comparisons	Mixture (1 major contributor) Major – consistent with: • Suspect – Neither the listed individual nor any of his paternal male relatives can be eliminated as the major source of the Y-STR DNA profile [in the sperm fraction, not attributed to X]. This Y-STR profile has been observed (X) times in the Y-Chromosome Haplotype Database of (Y) profiles and is not expected to occur more frequently than 1 in (Z) male individuals in the U.S. population[&]. The remainder of this mixture contains DNA that is not of sufficient quality for comparison to a standard from any individual.
Mixture with major and minor contributors	Mixture (2 contributors) Consistent with contributions from: • Suspect 1 – Neither the listed individual nor any of his paternal male relatives can be eliminated as the major source of the Y-STR DNA profile [in the sperm fraction, not attributed to X]. This Y-STR profile has been observed (X) times in the Y-Chromosome Haplotype Database of (Y) profiles and is not expected to occur more frequently than 1 in (Z) male individuals in the U.S. population[&]. • Suspect 2 – Neither the listed individual nor any of his paternal male relatives can be eliminated as the minor source of the Y-STR DNA profile [in the sperm fraction, not attributed to X]. This Y-STR profile has been observed (X) times in the Y-Chromosome Haplotype Database of (Y) profiles and is not expected to occur more frequently than 1 in (Z) male individuals in the U.S. population[&].

Exclusion statements	Suspect – excluded Suspect – not the major contributor Suspect 1, Suspect 2 – not the major contributor
Data not sufficient for inclusion	The Y-STR DNA profile is not sufficient for inclusion. Suspect – inconclusive [due to insufficient data/due to the number of contributors. Upon submission of [a consensual partner standard/any relevant elimination standards], additional Y-STR conclusions may be possible.

Situation	**Analytical Detail**
Extracts sent for Y amplification	DNA profiling was performed using PCR with the Y-filer Plus® STR kit on previously extracted samples from Item X (see report issued XXXXX).
Suspect standard for GF/Y and evidence profiles for Y amplification	DNA profiling was performed using PCR with the GlobalFiler® and the Yfiler® Plus STR kits on a sample from Item x and with the Y-filer® Plus STR kit on previously extracted samples from Item X (see report issued XXXXX).
GF and Y's run on evidence and standard, all issued under one report	DNA profiling was performed using PCR with the GlobalFiler® and the Yfiler® Plus STR kits on samples from Items x and y.
Standard run in GF but not Ys due to evidence run in Ys with no usable profile	DNA profiling was performed using PCR with the GlobalFiler® STR kit on a sample from Item x and with the Y-filer® Plus STR kit on previously extracted samples from Item X (see report issued XXXXX).
No Y profile for evidence and standard run in GF only. Do not include the standard in the table that is for Y-STR conclusions.	DNA profiling was performed using PCR with the GlobalFiler® STR kit on a sample from Item Y and with the Yfiler® Plus STR kit on previously extracted samples from Item X (see report issued XXXXX).

Footnotes	
Database used for Y-STR frequency calculations	& Based on the Y Chromosome Haplotype Database (www.yhrd.org, Release: # Updated [DATE]) using the 95% upper confidence interval
Caregiver/cohabitation	# The occurrence of incidental transfer of DNA between household members is well established. The circumstances surrounding the deposition of the male/caregiver/family member DNA herein cannot be determined based on DNA results.

26.8 References

Scientific Working Group on DNA Analysis Methods, 2014. Interpretation Guidelines for Y-Chromosome STR Typing

27 Report Examples

Report Examples – FB Only

27.1 Example 1

To: Ottawa Hills Police Department
Detective Bob Jones
2125 Richards Road

Ottawa Hills, OH 43604

Re: Rape
Subject: Robert Smith
Victim: Jane Doe

BCI Lab Number: 05-00000

Analysis Date: April 15, 2005
Issue Date: April 17, 2005

Agency Number: A100

Submitted by Officer Mark Johnson on March 3, 2005

1. Sexual Assault kit from Jane Doe
2. Shirt from Jane Doe
3. Hair brush from Jane Doe residence
4. Underwear from Jane Doe residence

Item	Conclusions
1 Sexual Assault kit – Jane Doe	
1.1 Vaginal slide	Semen identified
1.2 Vaginal swabs	Samples collected for DNA Analysis
1.3 Anal slide	Not examined
1.4 Anal swabs	No semen identified
1.5 Oral slide	Not examined
1.6 Oral swabs	No semen identified
1.7 Skin stain swabs	No semen identified Samples collected for DNA Analysis
1.8 DNA Standard - Jane Doe	Samples collected for DNA Analysis
1.9 Head hair standard	Not examined
1.10 Pubic hair standard	Not examined
1.11 Pubic hair combings	Not examined
1.12 Fingernail samples	Not examined
2 Shirt	Not examined
3 Hair brush	Not examined
4 Underwear	Positive for acid phosphatase* Amylase identified Samples collected for DNA Analysis
*Acid phosphatase is found in semen, saliva, vaginal secretions, and some bacteria	

Remarks

All samples will be retained by the laboratory until DNA testing is completed.

Please submit reference standards consisting of two oral swabs each from the suspect and the consensual partner.

Analytical Detail

An alternate light source was used to assist in detection of stains. Presumptive analysis for semen was performed using chemical testing. Presumptive analysis for seminal fluid, the liquid component of semen, was performed using immunological testing. Microscopic analysis was used for confirmation of semen.

Analysis for amylase, a component of saliva and other body fluids, was performed using a chemical test.

27.2 Example 2 (Optional Administrative Close Letter)

To:	Agency Detective 409 E. Street City, OH 45402-1374	BCI Laboratory Number:	15-12345
		Date:	December 4, 2015
		Agency Case Number:	15-123
Offense:	Crime		
Subject(s):	Robert Smith		
Victim(s):	Jane Thompson		

Submitted on December 1, 2015 by Detective:

1. Evidence item

Based on the current guidelines regulating the CODIS database and the information currently provided to the lab regarding the submitted item(s), any forensic DNA profile that may be developed is not eligible for database entry without additional required information.
DNA testing has not been performed and the item is being returned to your department. Please provide the selected information below to our agency and resubmit the above item(s) for testing.

- □ Synopsis/brief narrative of case facts to include:
  - Description of incident
  - Where the items of evidence were located
  - Who the items of evidence belong to
  - How the evidentiary items relate to the crime in question
- □ Reference standards from subject and any individuals for elimination
  - □ Victim
  - □ Individuals with known contact to the crime scene evidence
  - □ Other: _____

Please contact me by telephone or email if you have any questions.

_____

Robert Jones
Forensic Scientist
(330) 659-4600
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REPORT EXAMPLES—DNA

27.3 Example 3

To:	Police Department 123 Main Street Any Town, OH 12345	BCI Laboratory Number: Analysis Date: April 15, 2018	18-81236 Issue Date: April 17, 2018
Offense:	Rape	Agency Case Number:	2013-1235
Subject(s):	Frank Thompson, Sam Smith		
Victim(s):	Jane Doe		

Submitted on April 03, 2018 by John Smith:

1. Brown paper bag containing underwear from Jane Doe
2. One manila envelope containing known standard from Frank Thompson
3. One manila envelope containing known standard from Sam Smith
4. One manila envelope containing elimination standard from Joe Cook
5. One manila envelope containing known standard from Jane Doe

Items	DNA Conclusions
1 Underwear from Jane Doe	Positive for acid phosphatase activity*
1.1 Swabbing from Underwear	Mixture (3 major contributors) Major - Consistent with contributions from: • Jane Doe – expected contributor • Joe Cook – consent partner • Frank Thompson – The estimated proportion of the population that cannot be excluded as possible major contributors to the mixture of DNA profiles in the sperm fraction is 1 in 300,000^ unrelated individuals. Sam Smith - not a major contributor The remainder of the mixture contains DNA that is not of sufficient quality for comparison to a standard from any individual.
2 DNA standard – Frank Thompson	Profile used for comparison purposes
3 DNA standard – Sam Smith	Profile used for comparison purposes
4 DNA standard – Joe Cook	Profile used for comparison purposes
5 DNA Standard – Jane Doe	Profile used for comparison purposes
*Acid phosphatase is found in semen, saliva, vaginal secretions, and some bacteria ^ Based on the national database provided by the National Institute of Standards and Technology	

Remarks

Eligible DNA profiles (Items 1.1 and 3) have been entered into the CODIS database in accordance with state and national regulations, where regular searches will be performed. If investigative information becomes available or a profile is removed from CODIS, your agency will be notified.

Additional sample from each item is available should independent analysis be requested. All remaining items will be returned to the submitting agency. Any remaining DNA extracts will be retained by the laboratory.

Analytical Detail

An alternate light source was used to assist in detection of stains. Presumptive analysis for semen was performed using chemical testing.

DNA profiling was performed using PCR with the Globalfiler® STR kit on samples from Items 1-4.

27.4 Example 4

To:	Police Department 123 Main Street Any Town, OH 12345	BCI Laboratory Number: Analysis Date: April 15, 2018	18-81237 Issue Date: April 17, 2018
Offense:	Rape	Agency Case Number:	2013-1235
Subject(s):	Tom Smith		
Victim(s):	Jane Doe		

Submitted on April 03, 2018 by John Smith:

1. Sexual Assault kit from Jane Doe

Items	DNA Conclusions
1 Sexual Assault kit from Jane Doe	
1.1 Vaginal slide	Not examined
1.2 Vaginal swabs	Positive for acid phosphatase activity* No DNA profile foreign to Jane Doe[Y]
1.3 Anal/Perianal slides	Not examined
1.4 Anal/Perianal swabs	No DNA profile foreign to Jane Doe
1.5 Oral slide	Not examined
1.6 Oral swabs	No DNA profile foreign to Jane Doe
1.7 Underwear	Positive for acid phosphatase activity* Presumptive positive for blood
1.7.1 Cutting from stain in crotch	No DNA profile foreign to Jane Doe
1.7.2 Swab of inside front panel and crotch	Mixture (1 major contributor) Major – consistent with: • Jane Doe – expected contributor The remainder of the mixture contains DNA that is not of sufficient quality for comparison to a standard from any individual.
1.7.3 Swab of inside back panel	Mixture (1 major contributor) Major – consistent with: • Jane Doe – expected contributor The remainder of the mixture contains DNA, at least some portion of which is from a male, that is not of sufficient quality for comparison to a standard from any individual.[Y]
1.8 DNA standard	Profile used for comparison purposes

* Acid phosphatase is found in semen, saliva, vaginal secretions, and some bacteria

[Y] Sample eligible for Y-STR analysis

Remarks

Additional sample from each item is available should independent analysis be requested. All remaining items will be returned to the submitting agency. The remaining DNA extracts will be retained by the laboratory.

The forensic DNA profiles developed were not suitable for CODIS entry.

Y-STR analysis may be helpful in determining the source of any male DNA that is present. Submission of a reference standard consisting of two oral swabs from Tom Smith is required before analysis will proceed. Please ensure any available elimination standards are submitted.

_____

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Based on visual examination and scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

27.5 Example 5

To:	Police Department 123 Main Street Any Town, OH 12345	BCI Laboratory Number: Analysis Date: April 15, 2018
Offense:	Rape	Agency Case Number:
Subject(s):	Frank Thompson	
Victim(s):	John Smith	

Submitted on April 03, 2018 by John Smith:

1. White envelope containing blood card from John Smith
2. One manila envelope containing known standard from Frank Thompson (alleged father)
3. One manila envelope containing known standard from Sue Smith (mother)

Items	DNA Conclusions
1 DNA standard from John Smith	Profile used for comparison purposes
2 DNA standard from Frank Thompson	Profile used for comparison purposes
3 DNA standard from Sue Smith	Profile used for comparison purposes

Conclusions and Statistical Information

Frank Thompson cannot be excluded as being the biological father of John Smith.

Based on the national database provided by the National Institute of Standards and Technology, the evidence is 6,292,000,000,000,000,000 times more likely if Frank Thompson is the biological father of John Smith than if a random male is the biological father of John Smith.

Using a prior probability of 0.5, the probability that Frank Smith is the biological father of John Smith is 99.9999%.

Scale of Verbal Qualifiers for Likelihood Ratios	
Likelihood Ratio	**Verbal Qualifier**
1	Uninformative
2-99	Limited Support
100-9,999	Moderate Support
10,000-999,999	Strong Support
≥1,000,000	Very Strong Support

Remarks

The eligible DNA profile (Item 2) has been entered into the CODIS database in accordance with state and national regulations, where regular searches will be performed. If investigative information becomes available or a profile is removed from CODIS, your agency will be notified.

Additional sample from each item is available should independent analysis be requested. All remaining items will be returned to the submitting agency. Any remaining DNA extracts will be retained by the laboratory.

Analytical Detail

DNA profiling was performed using PCR with the Globalfiler® STR kit on samples from Items 1-3.

_____

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Based on visual examination and scientific analyses performed, this report contains opinions and interpretations by the analyst whose signature appears above. Examination documentation and any demonstrative data supporting laboratory conclusions are maintained by BCI and will be made available for review upon request.

27.6 Example 6

To:	Sheriff's Office	BCI Laboratory Number:	20-12345
	Det.	**Cross Reference Number:**	**15-12345**
	123 Main Street	Match Date:	Issue Date:
	Any Town, OH 12345	June 27, 2020	November 09, 2020
		Agency Case Number:	2013-1235
		BCI Agent:	

Offense: LINK
Subject(s):
Victim(s): John Doe

Submitted on March 26, 2020 by S/A:

1. One manila envelope containing tooth and a bone from John Doe
   1.1 Tooth

15-12345 Submitted on September 29, 2015 by Sergeant (Police Department):

1. One manila envelope containing standard from Jane Smith (mother of Joe Smith)

Missing Persons DNA Database Report

A search of the CODIS (Combined DNA Index System) database has resulted in an association between the following reference sample and remains sample (CODIS Rank ID DCR000012345):

Reference Sample

UNT Center for Human ID	3500 Camp Bowie Blvd, CBH-624, Fort Worth,TX 76107
Laboratory Specimen ID	UNTHSC-15-12341
Agency Submitting sample to UNT	Ohio Bureau of Criminal Investigation BCI 15-12345
Specimen Category	Biological Mother
Reference Item Name	Jane Smith
Reference for Missing Person	Joe Smith. (NamUs MP12345) Sex: Male DOB: 9/01/1990 Race: African-American Date of Last Contact: 10/01/2013

Remains

Ohio Bureau of Criminal Investigation	London Office – see report by Robert Jones issued July 7, 2020
Laboratory Specimen ID	20-212345 1.1 G127
Specimen Category	Unidentified Person (NamUs UP12345)
Item Type	Tooth

Kinship Conclusions and Statistical Information

Jane Smith (UNTHSC-15-12345.1) cannot be excluded as being the biological mother of the donor of the DNA from the tooth (BCI 20-12345 1.1). Based on the national database provided by the National Institute of Standards and Technology, the evidence is 13,500 times more likely if Jane Smith (UNTHSC-15-12345.1) is the biological mother of the donor of the DNA from the tooth (BCI 20-12345 1.1) than a random individual.

Given the genetic profile of the donor of the DNA from the tooth (BCI 20-12345 1.1), the probability of excluding a random female as the biological mother is 99.9999%.

Analytical Detail and Remarks

DNA profiling previously performed on a sample from BCI 20-12345 1.1 (see report issued July 7, 2020) was compared to the DNA profile entered into CODIS for Jane Smith (UNTHSC-15-12345.1/BCI 15-12345).

In addition to these genetic results, further investigation and/or review of additional case information may be required before declaring the identity of the human remains.

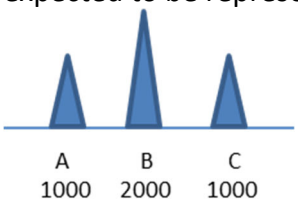
28 Appendix A – Globalfiler Interpretation Aid

Locus examples for CPI and RMP:

CPI Examples:

Unrestricted CPI Examples

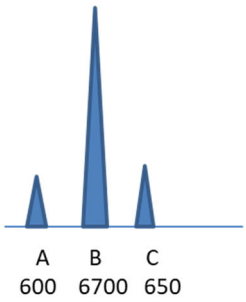
- All alleles are above the stochastic threshold of 600 RFUs and all contributors are expected to be represented.



A, B, and C used for unrestricted CPI statistic.

- A minor allele is present and above the stochastic threshold of 600 RFUs; however, the peak may be completely from stutter or a composite of a stutter product and a true allele. Not every peak in the stutter position should be considered as indistinguishable from stutter (e.g., two minor alleles present in non-stutter positions or reasonable to assume the minor data is from an expected contributor).

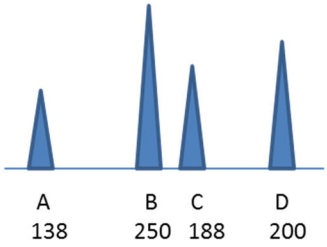
  See SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories, Approved 01/12/2017



Assuming a 10% stutter filter, the A would not be labeled. Given the RFU value of B, the A and C could be the genotype of the minor contributor. An unrestricted CPI may not be appropriate.

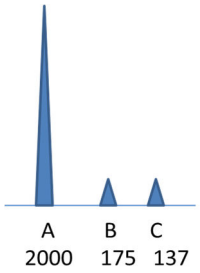
Restricted CPI Examples

- An assumption of a number of contributors is made and the maximum possible alleles are detected. For example, two contributors and 4 alleles called.



If assuming two contributors to profile, then use A,B,C,D for mixture calculation.

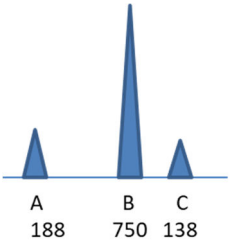
- A major profile is subtracted from the mixture and the remaining alleles represent the genotype of the additional contributor.



:

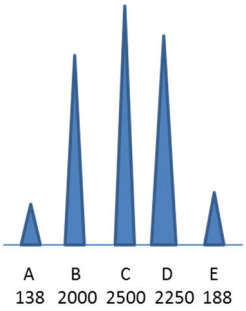
Major = A,A. If assuming two contributors, the second person must be B,C. Use locus as appropriate for the calculation.

- The victim or elimination profile is subtracted from the mixture and the remaining alleles represent the genotype of the additional contributor.



Victim = B,B. If assuming two contributors, the second person must be A,C. Use locus as appropriate for the calculation.

- A major mixture stat can be issued with clear distinction between the major and minor contributions. For example, the highest contribution from the minor contributor should be less than 40% of the lowest RFU allele of the major contribution.

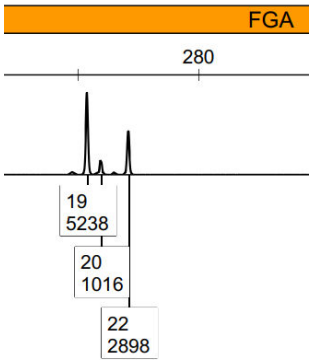
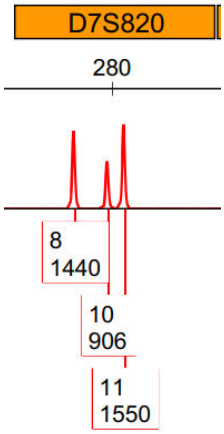


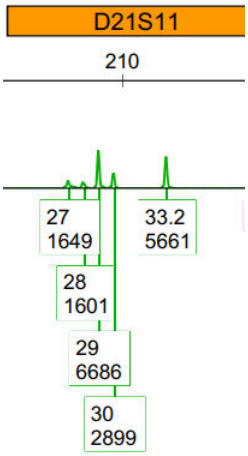
Use B,C,D in major mixture calculation because clearly distinct from the minor A,E alleles.

ArmedXpert Examples:

NOTE: DO NOT USE AN ANY (^) AND AN OBLIGATE (*) TOGETHER AT LOCI IN ArmedXpert.

Single Source Major:

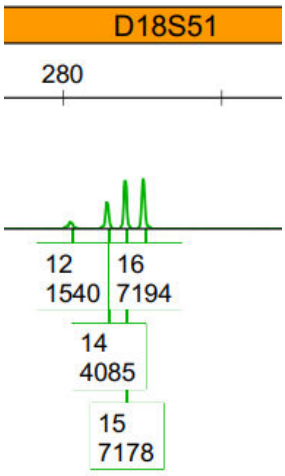
Qualifications	Example
In the event the PHR between the two largest peaks drops below 60%, but remains above 25%, obligate the tallest allele and click the next highest allele. The minor allele that is not considered as from the major contributor must be <40% of the lower major allele. If the PHR is >40%, perform a uRMP with the three alleles (assuming alleles >600 RFU). Tallest allele must be > 1200 RFU to be obligate.	 Tube 8, FGA 19*, 22 22/19 = 55.3% 20/22 = 35.0%
In the event there is a 3 peak pattern at a locus in a profile where a SS Major is being considered, apply stutter. If this does not decrease the peak to <40%, consider applying a uRMP at this location. Further clarification below.	 Tube 64, D7 10/8 = 62.9% 8,10,11 (all combos)



Example 1:
(5:1:1 Tube 121, Mix 2)

First evaluate the two highest peaks for a possible het major (33.2/29 = 84.6%). If they are > 60% PHR, then consider the 3rd highest peak to the 2nd highest peak to evaluate the 40% rule (30/33.2 = 51.2%). If the 40% rule is not met then consider doing a uRMP on the major group (29,30,33.2).

Then evaluate the smallest allele in the major group to the highest minor peak (27/30 = 56.9%). Remember, when calculating the 40% rule for a major group stutter is not considered. Therefore, this **does not meet** the 40% rule, and this location would be left out of the major statistic.

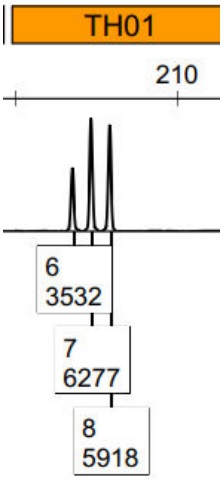


Example 2:
(5:1:1 Tube 121, Mix 2)

First evaluate the two highest peaks for a possible het major (15/16 = 99.7%). If they are > 60% PHR, then consider the 3rd highest peak to the 2nd highest peak to evaluate the 40% rule (14/15 = 56.9%/ 52.5% with stutter). If the 40% rule is not met then consider doing a uRMP on the major group (14,15,16).

Then evaluate the smallest allele in the major group to the highest minor peak (12/14 = 37.6%). Remember, when calculating the 40% rule for a major group stutter is not considered. Therefore, this **does meet** the 40% rule, and this location would be used in the major statistic.

When – stutter considered, 14 drops to 3304, 15 drops to 6285.

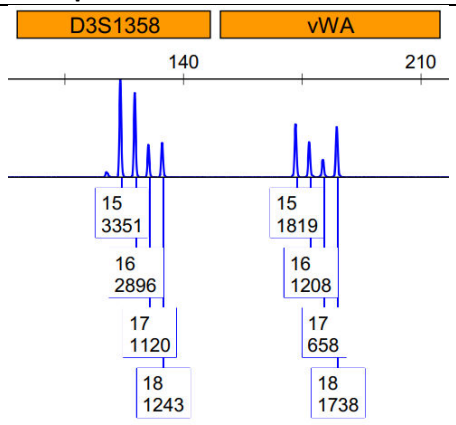


Example 3:
(5:1:1 Tube 121, Mix 2)

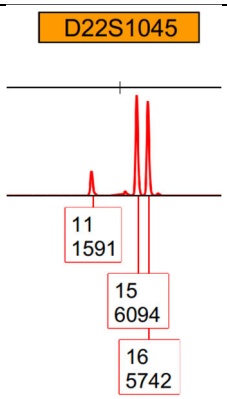
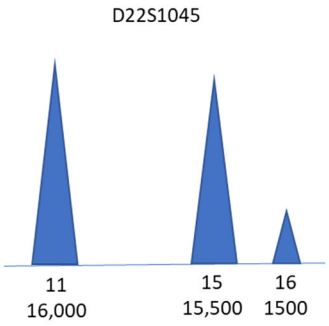
Only three alleles present and the major is well represented throughout the profile. Calculate a uRMP on 6, 7, 8.

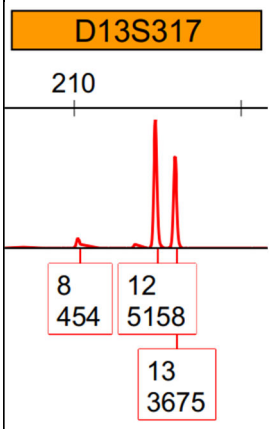
2p Mixtures – Deconvolution into Major/Minor Contributors

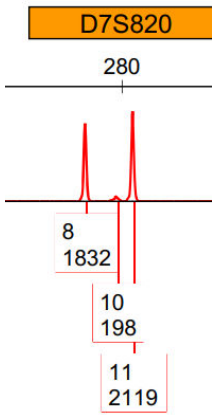
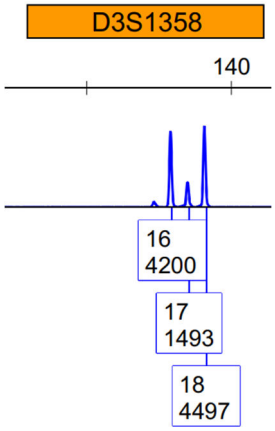
Four Peak Patterns:

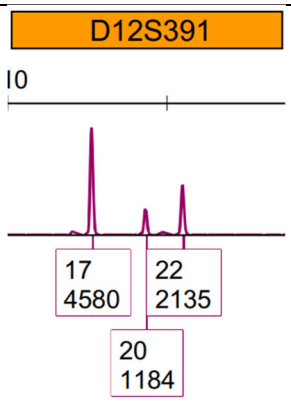
Qualifications	Example
Locus cannot be deconvoluted into major and minor contributors – 40% rule broken. While it may seem like you could eliminate combinations based off of the peak height ratios at these locations, do not. Approach: Choose all heterozygous combinations	 M2 2 to 1 (1.0ng) D3, vWA

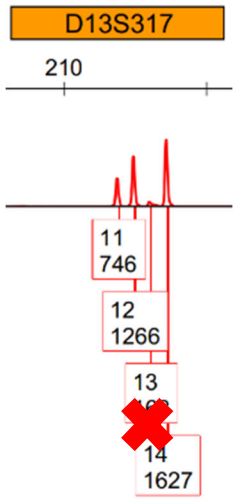
Three Peak Patterns:

Qualifications	Example
Overall good data All three peaks are above ST Major – 15,16 Minor – obligate the third allele 11*, 15, 16	 Tube 8 D02, D22
Major strong, above ST Minor peak is in stutter position Consider locus specific stutter <u>Approach:</u> Major → 11, 15 Minor → Does Allele drop below **<u>ST</u>**? - If yes, apply "allele, any" to minor allele. - If no, and the minor is not stochastic throughout, obligate the minor allele.	 16 at 1500 RFU goes down to 463 RFU after stutter applied. Designate minor type as 16, any.

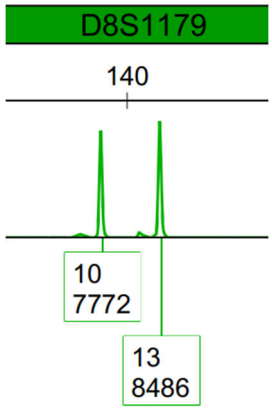
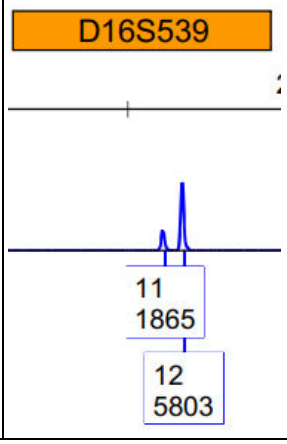
Qualifications	Example
Major strong, above ST Minor peak present, below ST <u>Approach:</u> Major → 12, 13 Minor → apply "allele, any" to minor allele	 Designate minor type as 8, any. Tube 21 A04, D13

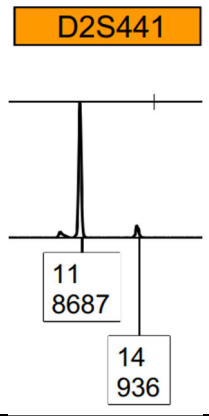
Major strong, above ST Minor allele in stutter position, below ST Consider locus specific stutter <u>Approach:</u> Major → 8, 11 Minor → Does allele drop below **<u>AT</u>**? - If yes, do not include this locus in minor statistic. - If no, apply "allele, any" to minor allele.	 Tube 4 H01, D7 10 goes down to 28 with stutter
Major strong, above ST Two major alleles, 1 minor allele in sandwich stutter Consider locus specific stutter <u>Approach:</u> Major → 16, 18 Minor → Does allele drop below **<u>ST</u>**? - If yes, apply "allele, any" to minor allele. - If no, obligate singular minor allele and choose the two major. (16, 17*, 18) Does allele drop below **<u>AT</u>**? - If yes, do not include this locus in minor statistic. - If no, apply "allele, any" to minor allele.	 Tube 3 G01, D3 17 at 1493 RFU goes down to 999 RFU after stutter applied. Note: In rare instances plus stutter could be considered when the major is very strong (approx. 10k+ RFU peaks).

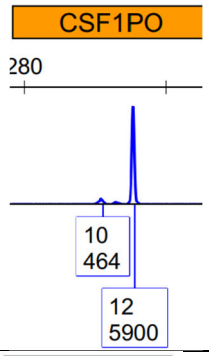
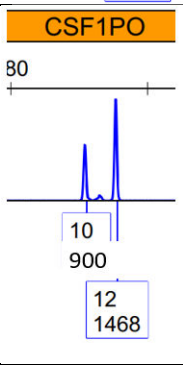
Qualifications	Example
Overall good data All peaks above ST No visible major/minor contributors Major → uRMP Minor → uRMP	 Tube 32 E05, D12

Major strong, above ST (some locations not clear) Minor stochastic, peak heights vary greatly Approach: Major → First apply general interpretation guidelines. If still unclear, calculate a uRMP or mRMP if one allele drops below ST when stutter considered. Minor → uRMP or can apply "allele, any" (mRMP) if one falls below ST	 Note: 13 considered elevated stutter Quant target less than 0.75ng Major is strong, minor stochastic and peak heights vary greatly 11 goes down to 630 after stutter applied

Two Peak Patterns:

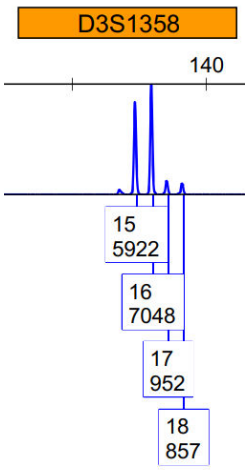
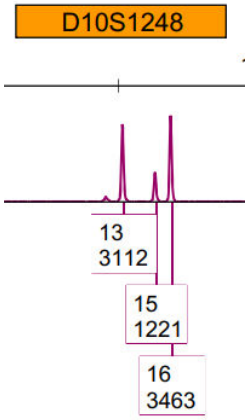
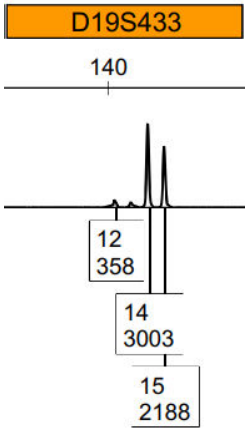
Qualifications	Example
Two peaks well above ST PHR > 60% All other minor peaks above ST throughout the profile Approach: Major → 10, 13 Minor → 10,13 OR uRMP • Dependent on analyst comfort with minor profile and contributor ratios throughout. Note: If minor is stochastic throughout profile, leave this location out of the minor statistic.	 Tube 20 H03, D8
Two peaks well above ST PHR < 40% 4x Rule not met All other minor peaks above ST throughout the profile, no concern for drop out of either contributor. Approach: Major → uRMP or 11,12* Minor → uRMP	 2 person 1ng A

All data above ST PHR < 40% Confident all data present (additional minor peaks at/above ST) ○ Major → 11 Minor → 11, 14*	 M2 5 to 1 (1.0ng) D2S441

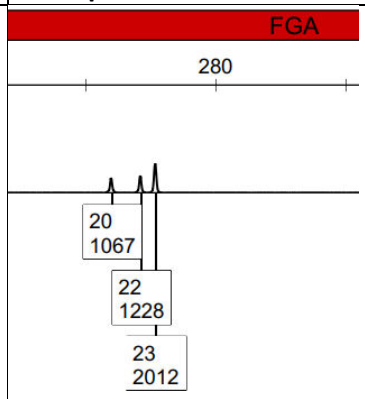
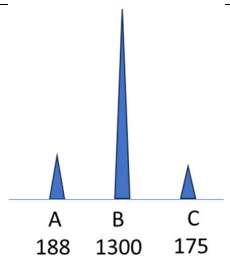
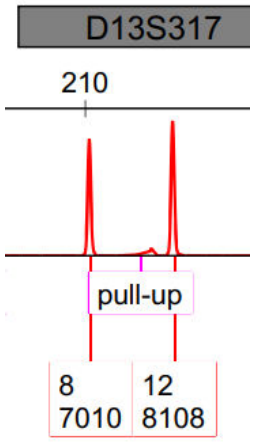
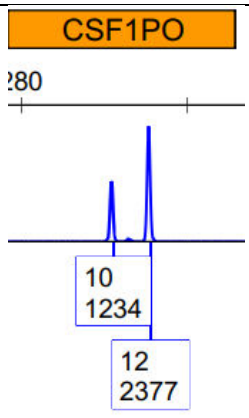
Qualifications	Example
One peak below ST PHR < 40% Confident Major is fully represented Minor may have drop out Approach○: Major → 12 Minor → 10, any	 M2 10 to 1 (1.0ng), CSF
Major is above ST, minor is stochastic Possible concern for drop out PHR > 60% Also applies when one peak is above ST and one is below ST. Approach: Major → use in statistic Minor → leave out	 M2 1 to 2 (0.5ng), CSF

○ In most situations, when you have a two-peak pattern in a two-person mixture, you need one peak that is distinguishable as the minor contributor to use the location in a minor statistic. Meaning, you need the PHR between the two peaks to be less than 40% to ensure the presence of at least one minor peak. Unless you have the epitome of a good quality profile, do not use the location in the statistic. Ideally, both contributors will be well above the stochastic range to use a one peak location in a 2p/3p statistic. It is preferred, there is one visible peak belonging to the second contributor present, or PHR less than 40% to use the location in the statistic.

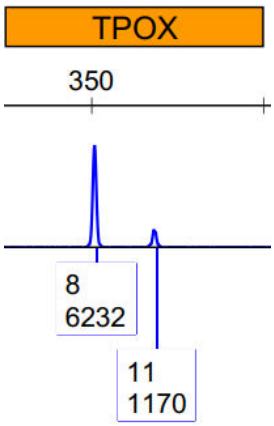
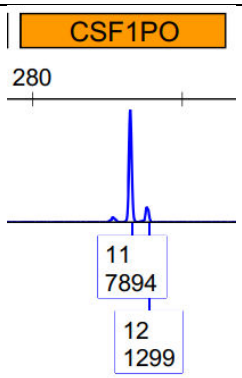
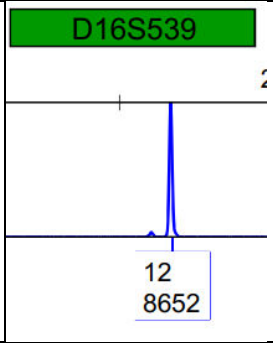
Deduced using Known:

Qualifications	Example	
Strong data No concern for drop out Two foreign peaks when EC/ stutter considered Approach: Assign genotypes		M2 10 to 1 (1.0ng) EC = 15, 16 DED = 17, 18
Strong data No concern for drop out Three peaks, all > 600RFU PHR for EC > 60% Single foreign allele Approach: Obligate foreign allele		M2 1 to 5 (1.0ng) EC = 13, 16 DED = 13, 15*, 16 Note: 15 is about 824 RFU when stutter is considered.
Three peaks, foreign allele < 600 RFU Approach: Apply “any” to foreign allele		M2 1 to 5 (1.0ng) EC = 14, 15 DED = 12 ^ Note: PHR 15/14 = 73%

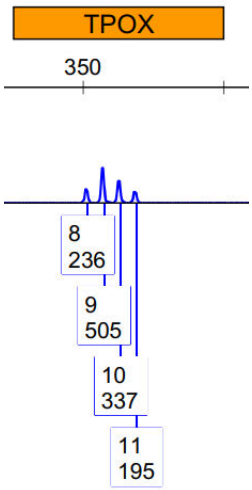
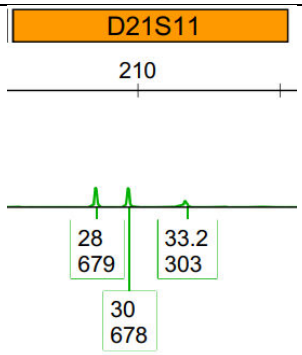
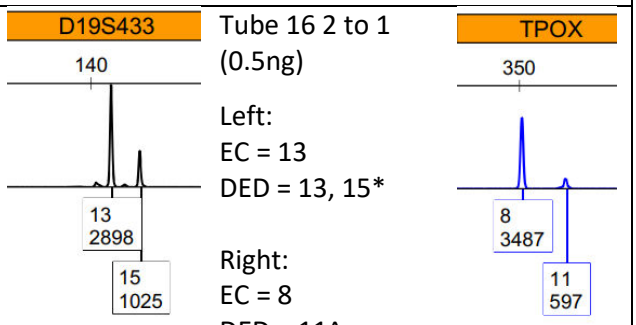
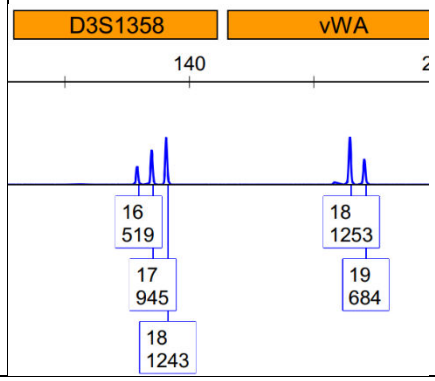
Deduced using Known (cont.)

Qualifications	Example
Strong data No concern for drop out Three peaks, all > 600RFU EC PHR < 60%, can infer sharing Approach: • Choose combo w/ sharing • Obligate singular foreign allele in combination with the remaining two alleles	 M2 2 to 1 (1.0ng) EC = 20,23 (53%) DED = 22, 23 Or 20,22*,23
3 peak pattern EC is A,C. DED can be B,B. (B>1200RFU)	
Strong data No concern for drop out Two peaks EC PHR > 60% No foreign allele Approach: Major → 8, 12 Minor →8, 12 OR uRMP • Dependent on analyst comfort with minor profile and contributor ratios throughout.	 Tube 14 1.0ng EC = 8, 12 DED = 8*, 12* Or 8, 12
Two peak location EC is hom (larger peak) No concern for drop out Approach: Consider ratio of contributors throughout profile, obligate foreign allele	 M2 1 to 1 (1.0ng) EC = 12 10/12 = 53% DED = 10*, 12

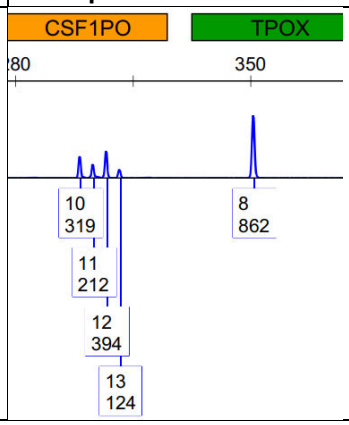
Deduced using Known (cont.)

Qualifications	Example
Two peak location EC is hom (smaller peak) No concern for drop out Approach: Profile should give indication using ratio as to whether or not unknown could be hom (big allele) at this location	 Tube 14 2 to 1 1.0 ng EC = 11 DED = 8 Note: contributor types picked as example, not truth data.
Two peak location No concern for drop out EC is het PHR < 60% Approach: choose higher allele as hom Note: Depending on the health of the overall profile and the ratios of the contributors, can also choose 11*, 12	 Tube 14 2 to 1 1.0ng EC = 11,12 DED = 11
Single peak No concern for drop out Approach: choose allele	 Tube 14 2 to 1 1.0ng EC = 12 DED = 12

Deduced using Known (cont.)

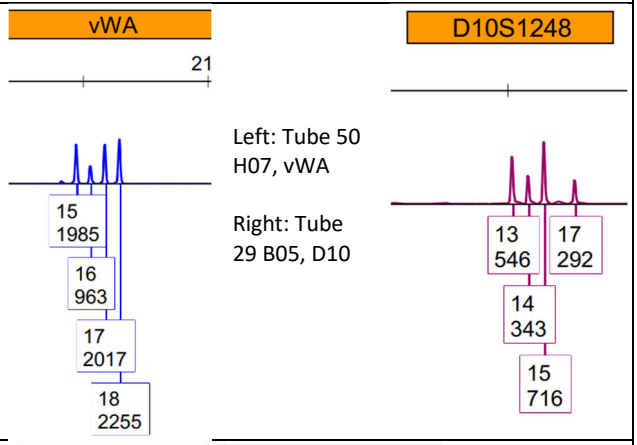
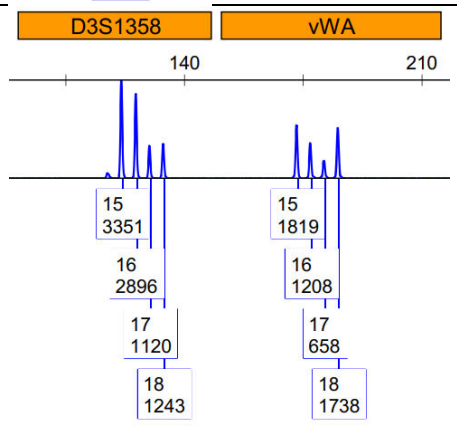
Qualifications	Example
Stochastic data Two foreign alleles >100 RFU after stutter Approach: Pick combo	 M2 2 to 1 (0.75ng) EC = 9, 10 DED = 8, 11
Stochastic data Three peak location Peaks at/near 600 RFU One foreign allele Approach: • Apply “any” to foreign allele • If locus is on the left hand side, not concerned about drop, could obligate foreign allele	 M2 2 to 1 (0.75ng) EC = 28, 30 DED = 33.2^ OR EC = 30, 33.2 DED = 28^
Stochastic data Two peak location PHR <60%, one foreign allele Approach: • Foreign allele > 600 RFU → obligate foreign • Foreign allele < 600 RFU → “allele, any” foreign allele	 Tube 16 2 to 1 (0.5ng) Left: EC = 13 DED = 13, 15* Right: EC = 8 DED = 11^
Stochastic data Two peak location PHR < 60%, no foreign allele Approach: Leave out	 Tube 17 2 to 1 (0.25ng) vWA EC= 18, 19 (55%) DED: Leave out

Deduced using Known (cont.)

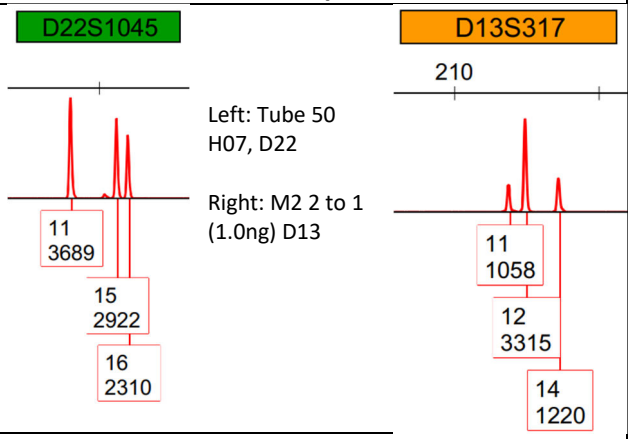
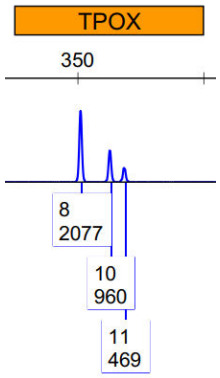
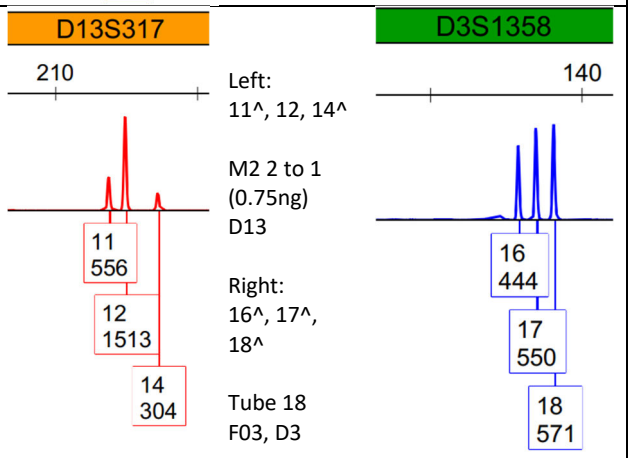
Qualifications	Example
Stochastic data Singular peak No foreign alleles Approach: Would need to be absolutely sure unknown is there before using. In a stochastic profile, best to leave out.	 Tube 35 2:1 (0.25ng) TPOX EC: 8 DED: Leave out Note: DED truth data is 8, 11

2p Mixtures – <u>NO</u> Deconvolution into Contributors

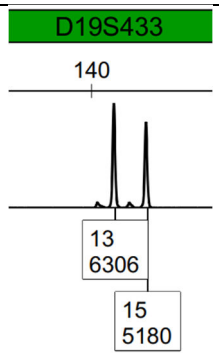
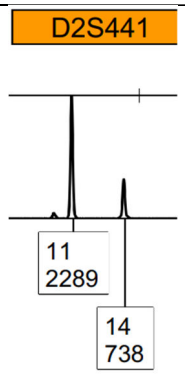
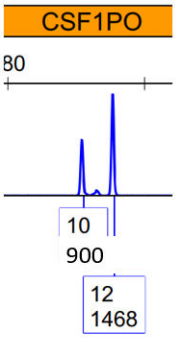
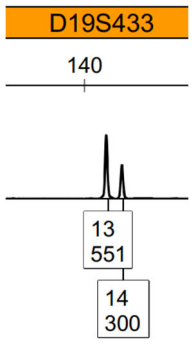
Two Person Mixture - Four Peak Patterns:

Qualifications	Example
Profile cannot be deconvoluted into major and minor contributors at most locations. Locus has the maximum number of alleles for the assumed number of contributors. Approach: consider all heterozygous genotypes (uRMP)	 Left: Tube 50 H07, vWA Right: Tube 29 B05, D10
Profile cannot be deconvoluted into major and minor contributors at most locations. While it may seem like you could eliminate combinations based off of the peak height ratios at these locations, do not. Approach: consider all heterozygous combinations (uRMP)	 M2 2 to 1 (1.0ng) D3, vWA

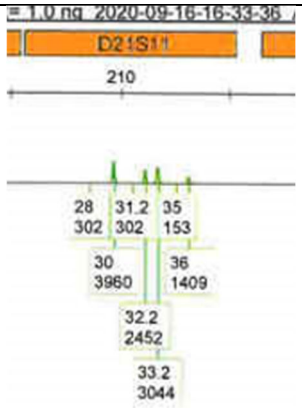
Two Person Mixture - Three Peak Patterns:

Qualifications	Example
All alleles above ST Approach: consider all allele combinations (uRMP)	 Left: Tube 50 H07, D22 Right: M2 2 to 1 (1.0ng) D13
One allele below ST Consider drop out Approach: calculate a modified RMP mRMP – will apply "allele, any" approach to allele below ST only.	 8, 10, 11^
Limited use Approach Profile characteristics: • Two person mixture (confident in #) • Profile straddles ST throughout • N-1 alleles present at the locus for the assumed number of contributors • 2-3 alleles below ST Approach: apply "allele, any" to alleles below ST Caution: The use of a location like this could inflate an overall stochastic profile where it is hard to assume the number of total contributors.	 Left: 11^, 12, 14^ M2 2 to 1 (0.75ng) D13 Right: 16^, 17^, 18^ Tube 18 F03, D3

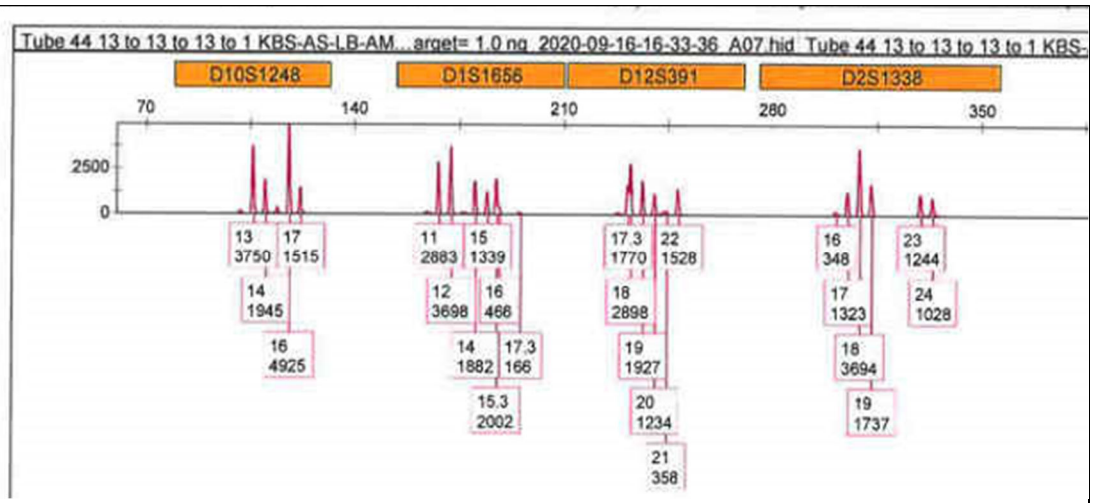
Two Person Mixture – Two Peak Patterns:

Qualifications	Example
Input is high Good quality data No concern for drop out Approach: consider all allele combinations (uRMP)	 Tube 2 F01, D19
Overall profile is above ST No concern for drop out PHR < 40% Approach: consider all allele combinations (uRMP)	 M2 1 to 2 (0.5ng), D2S441
Data to left and right of locus near ST Possible concern for drop out PHR > 60% Also applies when one peak is above ST and one is below ST. Approach: leave this locus out of the statistic, as you could be losing one of the contributors	 M2 1 to 2 (0.5ng), CSF
Input is low Not good quality data Concern for drop out (alleles close to/ below ST) Approach: leave this locus out of the statistic	 Tube 29 G02, D19

Two Person Major – Four Contributors

Major alleles of 30, 32.2, 33.2, 36 Two major contributors, clear separation from minor portion Approach: Het combos only, 30, 32.2 30, 33.2 30, 36 32.2, 33.2 32.2, 36 33.2, 36 If assume a major contributor (Known 30, 32.2), can use 33.2, 36.	

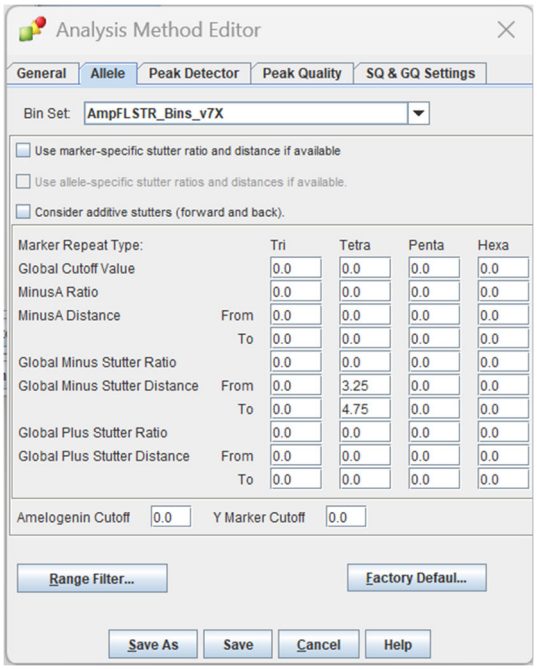
Three Person Major – Four Contributors

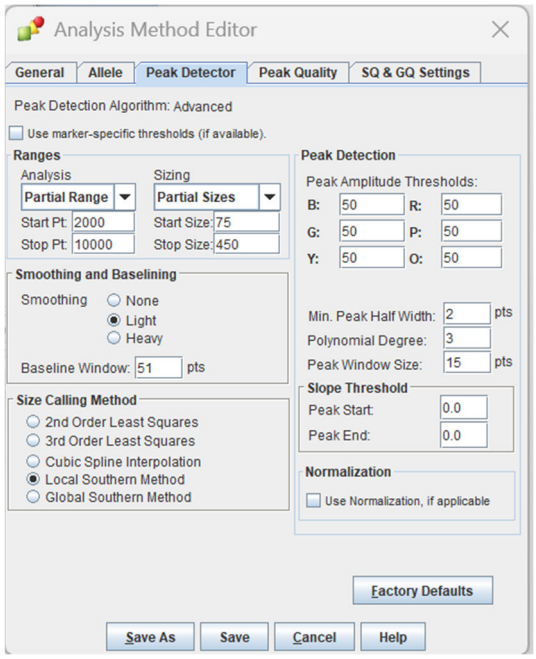
Clear separation of three major contributors from the minor uRMP D10 13, 14, 16, 17 D1 11, 12, 14, 15, 15.3 D12 17.3, 18, 19, 20, 22 D2 17, 18, 19, 23, 24	

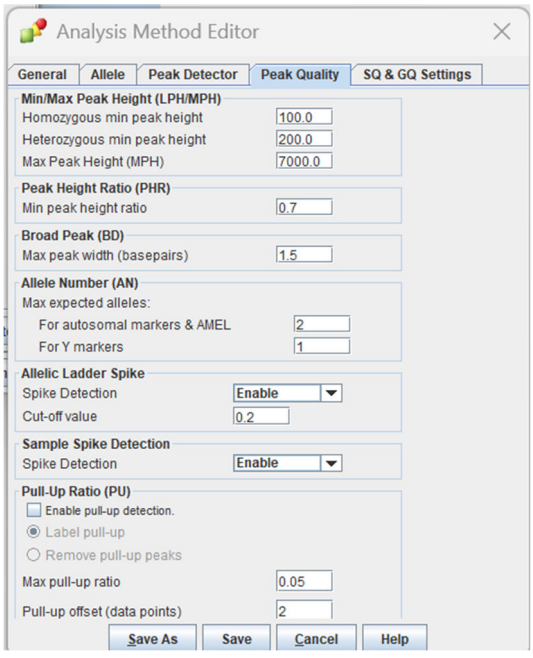
29 Appendix B Legacy Data Procedures

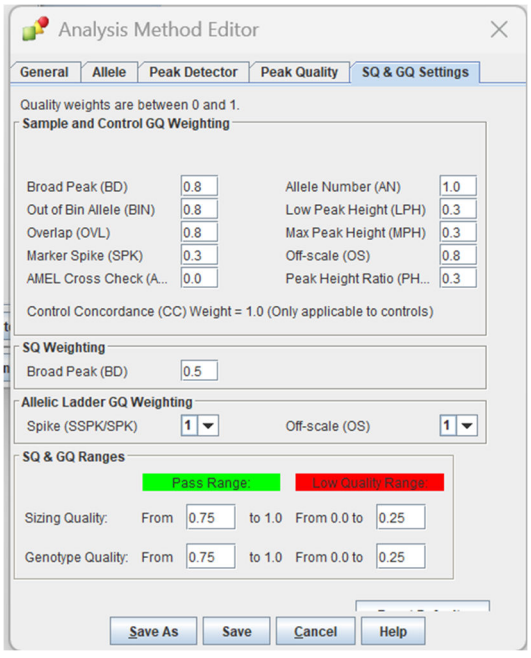
29.1 BCI Identifiler Legacy Genemapper v1.7 Settings

1. Identifiler analysis settings:
   - Analysis method: BCI Identifiler
   - Panel: Identifiler_v1.6X
   - Size Standard: CE_G5_HID_GS500
   - Bin Set: AmpFLSTR_Bins_v7x
   - Stutter ratio: Do not use marker specific (specific stutter values are loaded into the Panel manager)
   - Global Cut-off Value: 0.0 for all Marker Repeat Types
2. The screenshots below demonstrate the correct settings within the Analysis method.









29.2 Miami Valley Regional Crime Lab (MVRCL) Legacy Data Procedures (Updated for Genemapper IDX v1.7)

1. DNA Extracts
   DNA extracts from the MVRCL may be tested by BCI. Refer to section 17 of the DNA Methods manual to begin testing procedures. The reagent blank associated with the MVRCL extract must be tested by BCI and meet the requirements set forth in the DNA QA manual section 9.5.1 and the DNA Methods manual in order to report additional STR kit results for MVRCL DNA extracts. Extracts and blanks will be entered into LIMS as items received. The chain of custody for the MVRCL DNA extracts and reagent blanks will be tracked in the case documentation.
2. Data Re-analysis
   Data generated by the MVRCL may be re-analyzed using GeneMapper v1.4 software. For each amplification kit, refer to the DNA Methods manual unless addressed below. For MVRCL legacy data comparisons, random match probabilities (RMP) for single source and major single source profiles and restricted/unrestricted combined probability of inclusion (rCPI and CPI) for mixtures will be issued using PopStats.

The following situations are NOT considered a re-interpretation:

- The generation of a letter for the comparison of 2 samples as a result of a CODIS high stringency match is not considered reinterpretation of legacy data.
- If a casework (forensic) DNA profile interpretation has been previously documented regarding the genotypes that would be allowed for possible contributors, that interpretation is not considered re-interpretation.
- The import of a MVRCL .fsa or .hid file into GeneMapper ID-X to compare only the allele calls to the CODIS entry is not considered re-interpretation, but an administrative check.

The following situations are considered re-interpretation:

- Assessing/reevaluating allele calls and/or genotype calls (to include RFU values, potential allelic drop-out, number of contributors) for MVRCL data.
- Changing the assumptions used for MVRCL data.
- Adding allele/locus to an existing CODIS entry.
- Removing alleles/loci from an existing CODIS entry.
- Adding obligate allele indicators (+) to an existing CODIS entry.

Fusion Amplification Kit

Re-analyzing MVRCL Fusion with GeneMapper IDX software

1. Review the MVRCL case information to determine which run contains the .hid files for the samples and controls for reanalysis. Import the sample, controls, and ladder files into GeneMapper IDX.
2. Fusion analysis settings:
   Analysis method: MV Fusion 6C 24s or 40s
   Panel: PowerPlex_Fusion_6C_Panels_IDX_v1.2
   Size Standard: WEN_ILS_500_CS
   Bin Set: PowerPlex_Fusion_6C_Bins_IDX_v1.2
   Stutter ratio: Marker specific (specific stutter values are loaded into the Panel manager)

Global Cut-off Value: 0.0 for all Marker Repeat Types

3. The screenshots below demonstrate the correct settings within the Analysis method for 24s Fusion injections. The dye thresholds will be 175 RFU for the 40s injection time (except for ILS).
4. Turn on the marker-specific stutter filters for N-2, N-3, N-4, N+3 and N+4.

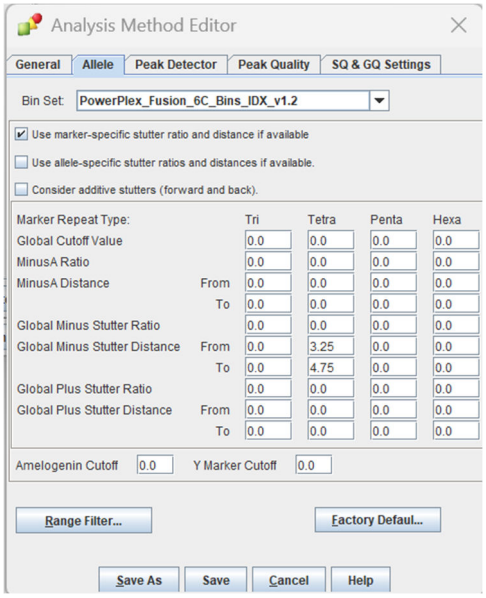
Fusion N-2, N-3, N-4, N-5 Stutter Filter % Values

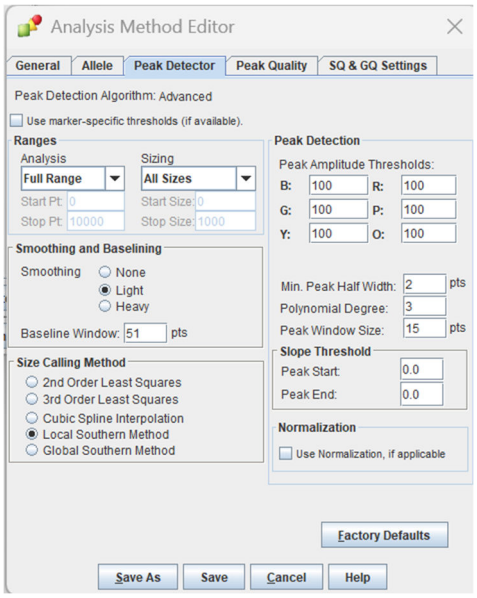
D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E
13.5	14.3/3.6 (N-2)	9	13	10.3	7.2
D16S1539	**D18S51**	**D2S1338**	**CSF1PO**	**Penta D**	
12	14.6	13.6	11.1	4.5	
TH01	**vWA**	**D21S11**	**D7S820**	**D5S818**	**TPOX**
4.8	14.4	12.7	9.7	11	5.4
D8S1179	**D12S391**	**D19S433**	**SE33**		
11.8	17.4	12.1/1.4 (N-2)	16.1/6.6 (N-2)		
D22S1045	**DYS391**	**FGA**	**DYS576**	**DYS570**	
16.8	9.4	12.4/1.2 (N-2)	12.5	13	

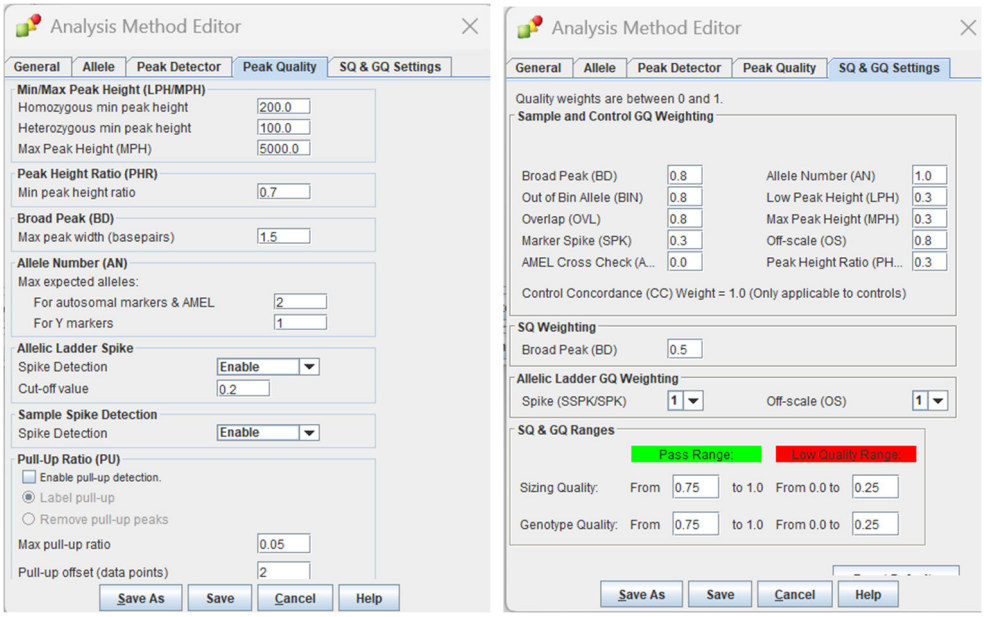
Fusion N+3, N+4, N+5 Stutter Filter % Values

D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E
1.7	2.3	1.8	1.3	2.2	1.9
D16S1539	**D18S51**	**D2S1338**	**CSF1PO**	**Penta D**	
3	2.8	2.2	3.7	3.7	
TH01	**vWA**	**D21S11**	**D7S820**	**D5S818**	**TPOX**
1.5	2.7	2.8	1.8	2.3	1.1
D8S1179	**D12S391**	**D19S433**	**SE33**		
3.4	2.7	2.6	3.3		
D22S1045	**DYS391**	**FGA**	**DYS576**	**DYS570**	
9	2	2.8	3.4	2.4	

5. The analysis range can be changed from “full range” to a more “partial range” to avoid the primer front and/or if sizing issues arise with the LIZ. Sizing needs to include the 60 bp peak as well as all of the other LIZ peaks through 500 bp to ensure correct sizing results.

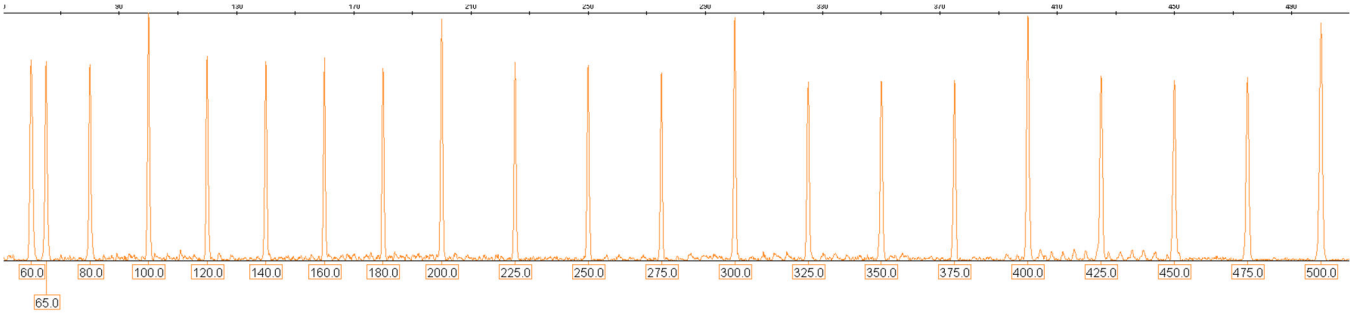






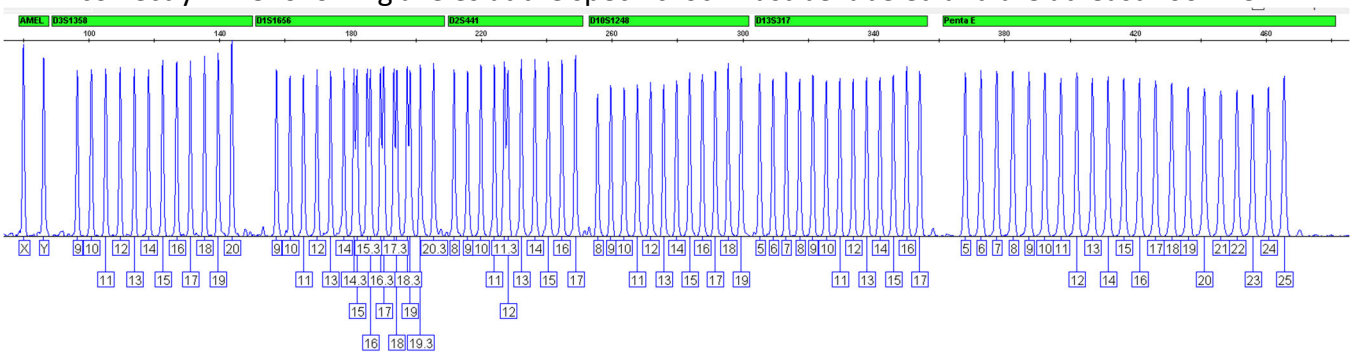
Assessment of Internal Lane Standard

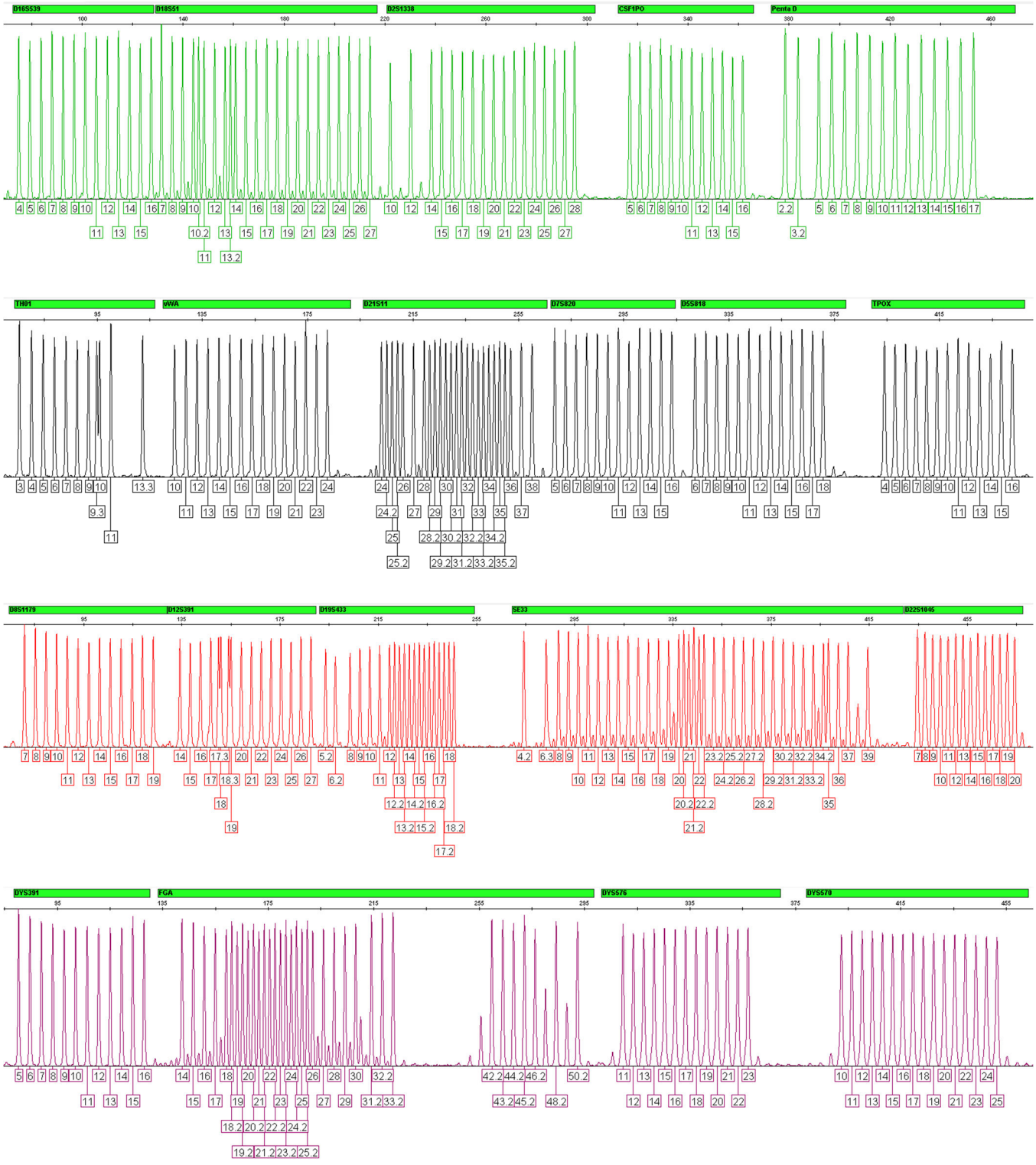
The WEN_ILS_500_CS internal size standard must have the expected size results for the 60.0, 65.0, 80.0, 100.0, 120.0, 140.0, 160.0, 180.0, 200.0, 225.0, 250.0, 275.0, 300.0, 325.0, 350.0, 375.0, 400.0, 425.0, 450.0, 475.0, and 500.0 base pair fragments. The peaks must be sizable but do not have to be above 100 RFU.



Assessment of Allelic Ladder

Examine the allelic ladders to determine that the software has assigned all allele designations correctly. The following alleles at the specific loci must be labeled and are at least 100 RFU.





Assessment of Controls

1. Amplification negatives and manipulation blanks should contain no profiles. When only a single spurious peak, peaks consistent with cross-talk, or known artifacts are present, no further troubleshooting is required and data analysis may be performed. Where peaks above the 100 RFU (24s injection) or 175 RFU (40s injection) analytical thresholds are present, bring

to the TL. Potential courses of action include interpretation of a reduced number of loci or reporting the sample as inconclusive.

2. Amplification positives should contain the profile as given in the table below since these samples were previously reported by the MVRCL.
3. Amplification Positive Control Types:

Fusion 6C	Kit Positive
Amel.	X, Y
D3S1358	17, 18
D1S1656	12, 13
D2S441	10, 14
D10S1248	13, 15
D13S317	9, 11
Penta E	7, 14
D16S1539	9, 13
D18S51	16, 18
D2S1338	22, 25
CSF1PO	12
Penta D	12, 13
TH01	6, 9.3
vWA	16, 19
D21S11	29, 31.2
D7S820	8, 11
D5S818	12
TPOX	11
D8S1179	14, 15
D12S391	18, 23
D19S433	13, 14
SE33	15, 16
D22S1045	16
DYS391	10
FGA	20, 23
DYS576l	18
DYS570	17

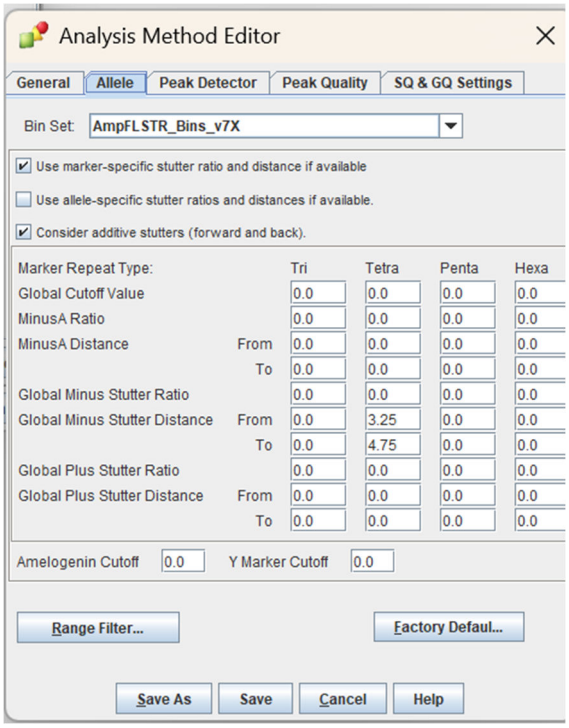
Fusion Data Interpretation

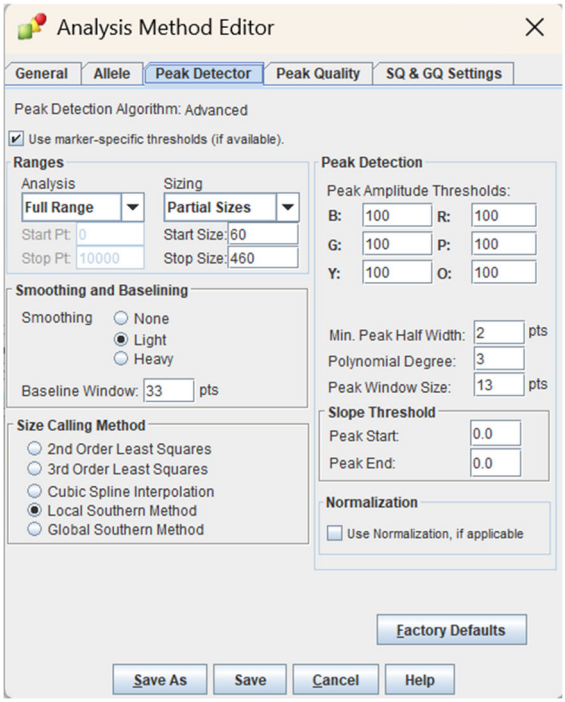
AT	24s 100 RFU, 40s 175 RFU
ST	24s 800 RFU, 40s 1100 RFU
PHR	High quality data ~60%
Major:Minor	Min. 40% difference
Minimum to interpret SS	Min. 1 but overall quality of data considered. RMP more common than 1 in 300 are reported as inconclusive.
Minimum to interpret SS maj	5 loci for 2 person; 10 loci for 3, 4 person
Minimum to interpret Mixed Major	5 loci for 2 person; 10 loci for 3, 4 person
Homozygote rule in mixtures	1200 RFU and ≥4x largest minor peak

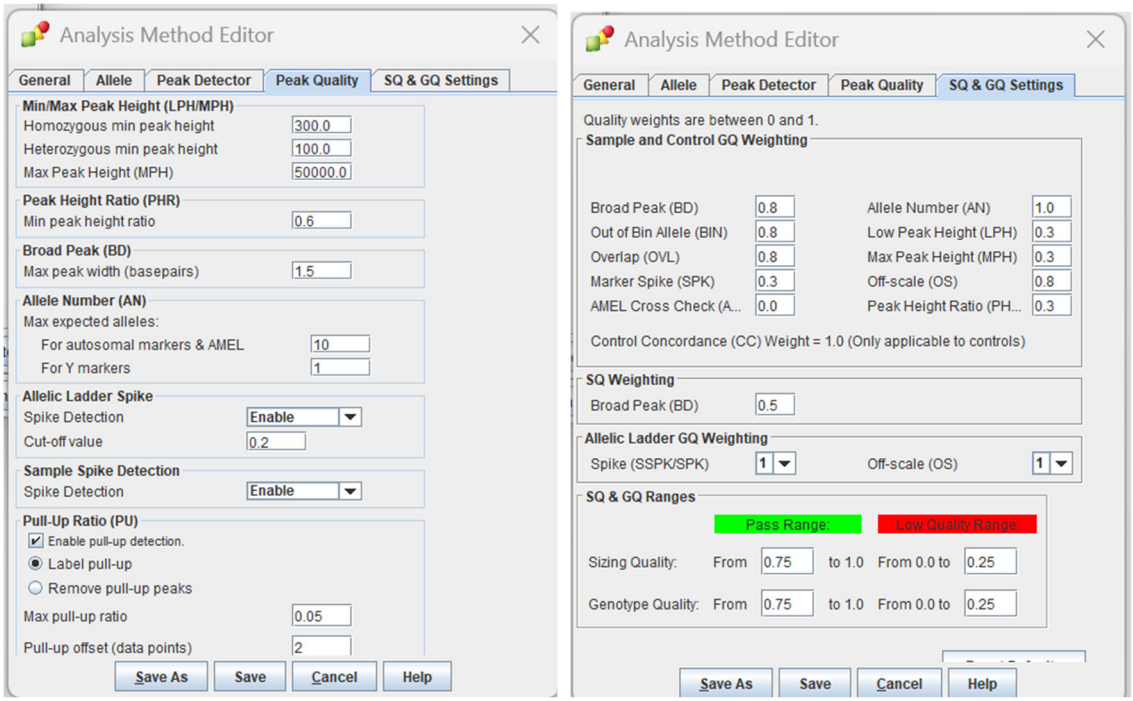
Globalfiler Amplification Kit

Re-analyzing MVRCL Globalfiler with Genemapper IDX software

1. Review the MVRCL case information to determine which run contains the .hid files for the samples and controls for reanalysis. Import the sample, controls, and ladder files into GeneMapper IDX.
2. Globalfiler analysis settings:
   Analysis method: Globalfiler 100 RFU
   Panel: Globalfiler_Panel_v1.2X
   Size Standard: GS600_LIZ_(60-460)
   Bin Set: AmpFLSTR_Bins_v7X
   Stutter ratio: Marker specific (specific stutter values are loaded into the Panel manager)
   Global Cut-off Value: 0.0 for all Marker Repeat Types
3. The screenshots below demonstrate the correct settings within the Analysis method.
4. Turn on the marker-specific stutter filters for N-2, N-3, N-4, N+3 and N+4.
5. The analysis range can be changed from “full range” to a more “partial range” to avoid the primer front and/or if sizing issues arise with the GS600 LIZ. The first allele bin is located after the 60 bp peak and is located at D2S441. Sizing needs to include the 60 bp peak as well as all of the other LIZ peaks through 460 bp to ensure correct sizing results.

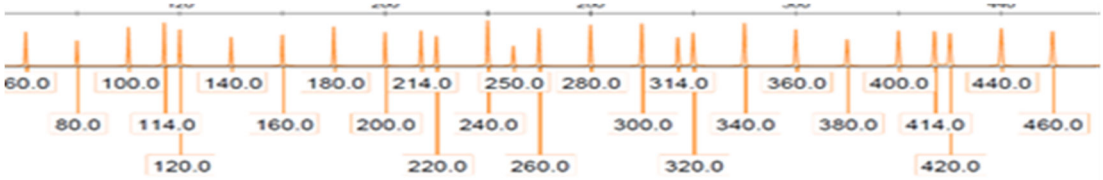






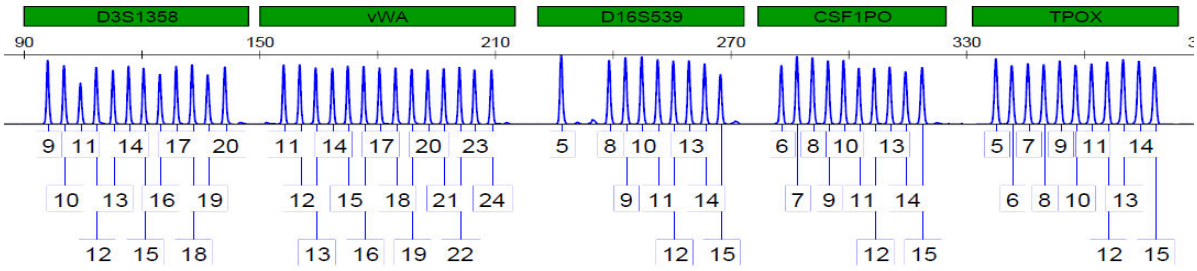
Assessment of Internal Lane Standard

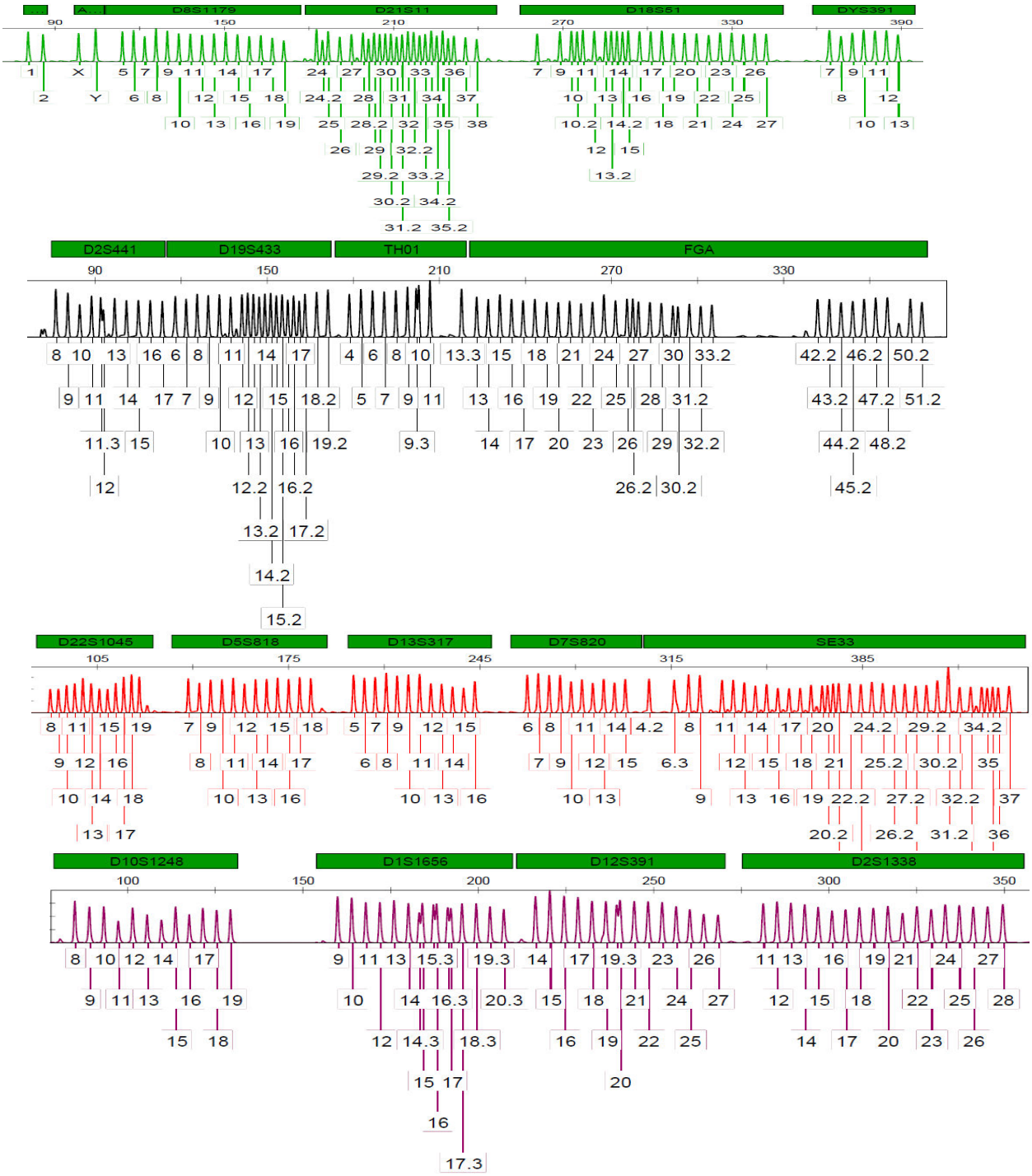
The LIZ 600 internal size standard must have the expected size results for the 60.0, 80.0, 100.0, 114.0, 120.0, 140.0, 160.0, 180.0, 200.0, 214.0, 220.0, 240.0, 250.0, 260.0, 280.0, 300.0, 314.0, 320.0, 340.0, 360.0, 380.0, 400.0, 414.0, 420.0, 440.0, and 460.0 base pair fragments. The peaks must be sizable but do not have to be above 100 RFU.



Assessment of Allelic Ladder

Examine the allelic ladders to determine that the software has assigned all allele designations correctly. The following alleles at the specific loci must be labeled and are at least 100 RFU.





Assessment of Controls

1. Amplification negatives and manipulation blanks should contain no profiles. When only a single spurious peak, peaks consistent with cross-talk, or known artifacts are present, no further troubleshooting is required and data analysis may be performed. Where peaks above the 100 RFU analytical threshold are present, bring to the TL. Potential courses of action include interpretation of a reduced number of loci or reporting the sample as inconclusive.
2. Amplification positives should contain the profile as given in the table below since these samples were previously reported by the MVRCL.

3. Amplification Positive Control Types:

Globalfiler	Kit Positive 007
D3S1358	15, 16
vWA	14, 16
D16S1539	9, 10
CSF1PO	11, 12
TPOX	8
Y-Indel	2
Amel.	X, Y
D8S1179	12, 13
D21S11	28, 31
D18S51	12, 15
DYS391	11
D2S441	14, 15
D19S433	14, 15
TH01	7, 9.3
FGA	24, 26
D22S1045	11, 16
D5S818	11
D13S317	11
D7S820	7, 12
SE33	17, 25.2
D10S1248	12, 15
D1S1656	13, 16
D12S391	18, 19
D2S1338	20, 23

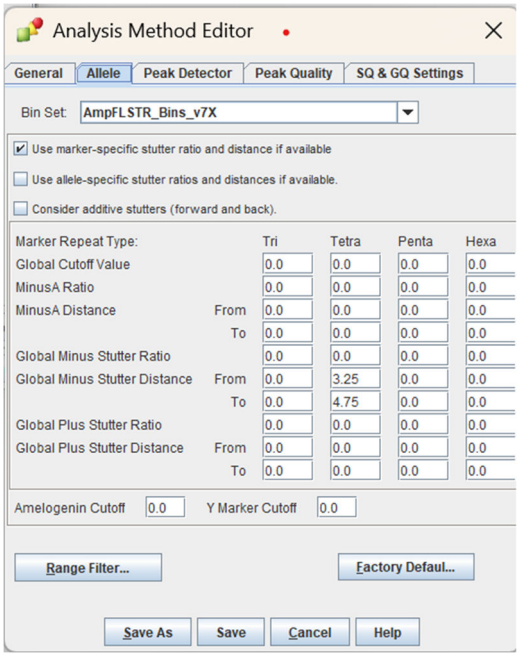
Globalfiler Data Interpretation

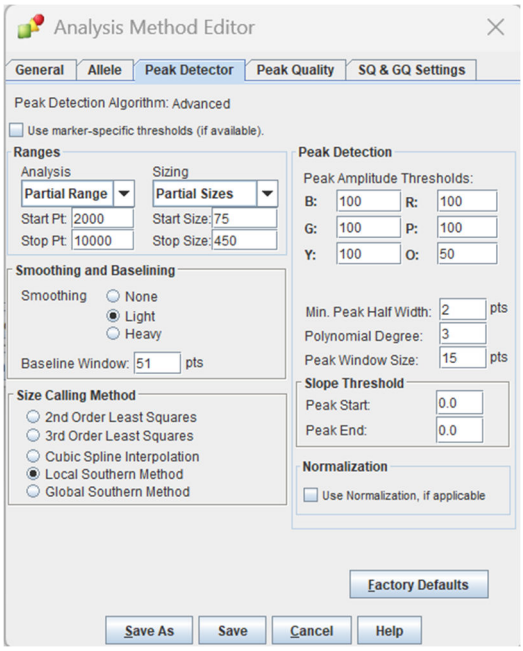
AT	100 RFU
ST	200 RFU
PHR	High quality data ~65%
Major:Minor	Min. 40% difference
Minimum to interpret SS	Min. 1 but overall quality of data considered. RMP more common than 1 in 300 are reported as inconclusive.
Minimum to interpret SS maj or 2 person	5 loci for 2 person; 10 loci for 3, 4 person
Minimum to interpret 3,4,5 person	5 loci for 2 person; 10 loci for 3, 4 person
Homozygote rule in mixtures	1200 RFU and ≥4x largest minor peak

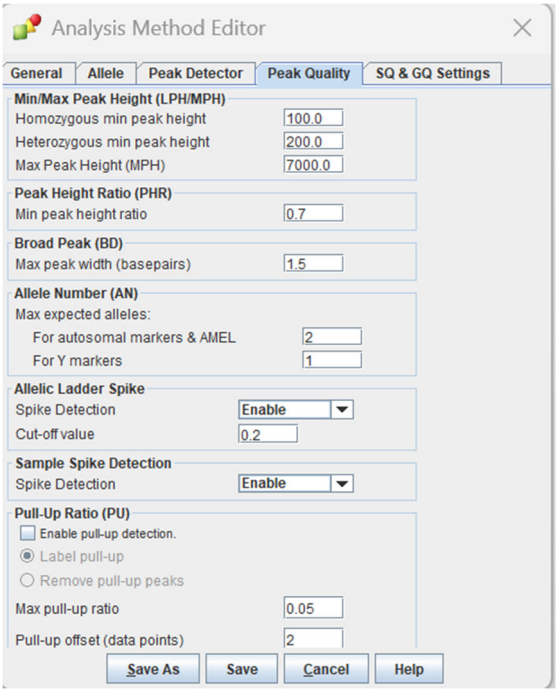
Identifiler Amplification Kit

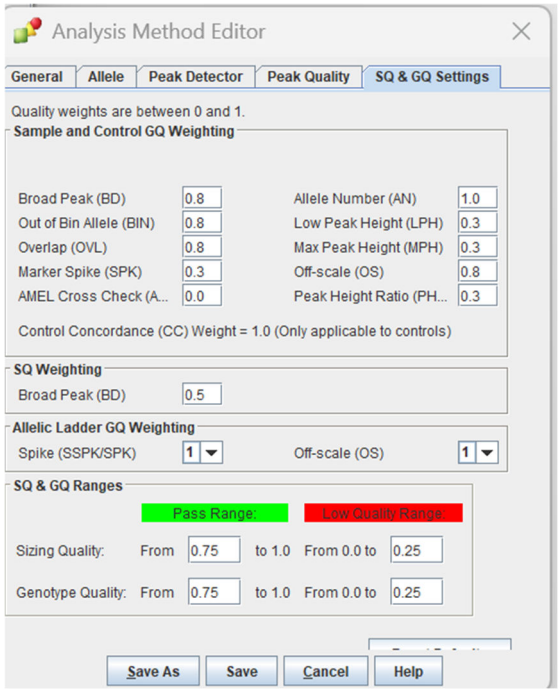
Re-analyzing MVRCL Identifiler with Genemapper IDX software

1. Review the MVRCL case information to determine which run contains the .hid files for the samples and controls for reanalysis. Import the sample, controls, and ladder files into GeneMapper IDX.
2. Identifiler analysis settings:
   Analysis method: MV Identifiler 100 RFU
   Panel: Identifiler_v1.6X
   Size Standard: CE_G5_HID_GS500
   Bin Set: AmpFLSTR_Bins_v7x
   Stutter ratio: Do not use marker specific (specific stutter values are loaded into the Panel manager)
   Global Cut-off Value: 0.0 for all Marker Repeat Types
3. The screenshots below demonstrate the correct settings within the Analysis method.
4. Turn off the marker-specific stutter filters.
5. The analysis range can be changed from “full range” to a more “partial range” to avoid the primer front and/or if sizing issues arise with the LIZ. Sizing needs to include the 75 bp peak as well as all of the other LIZ peaks through 450 bp to ensure correct sizing results.



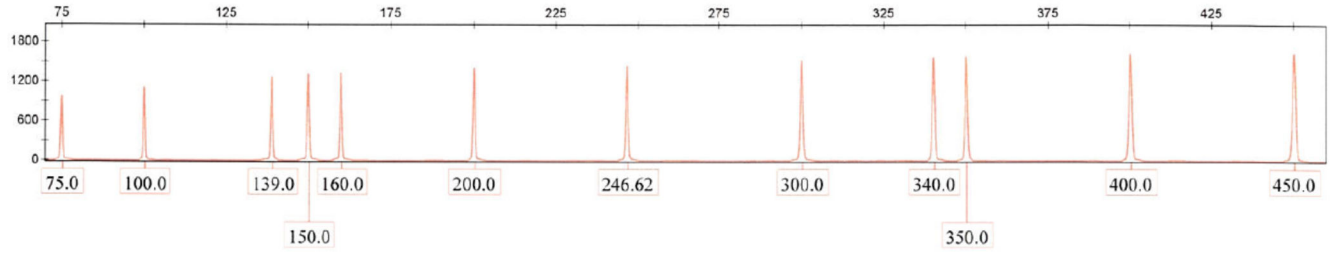






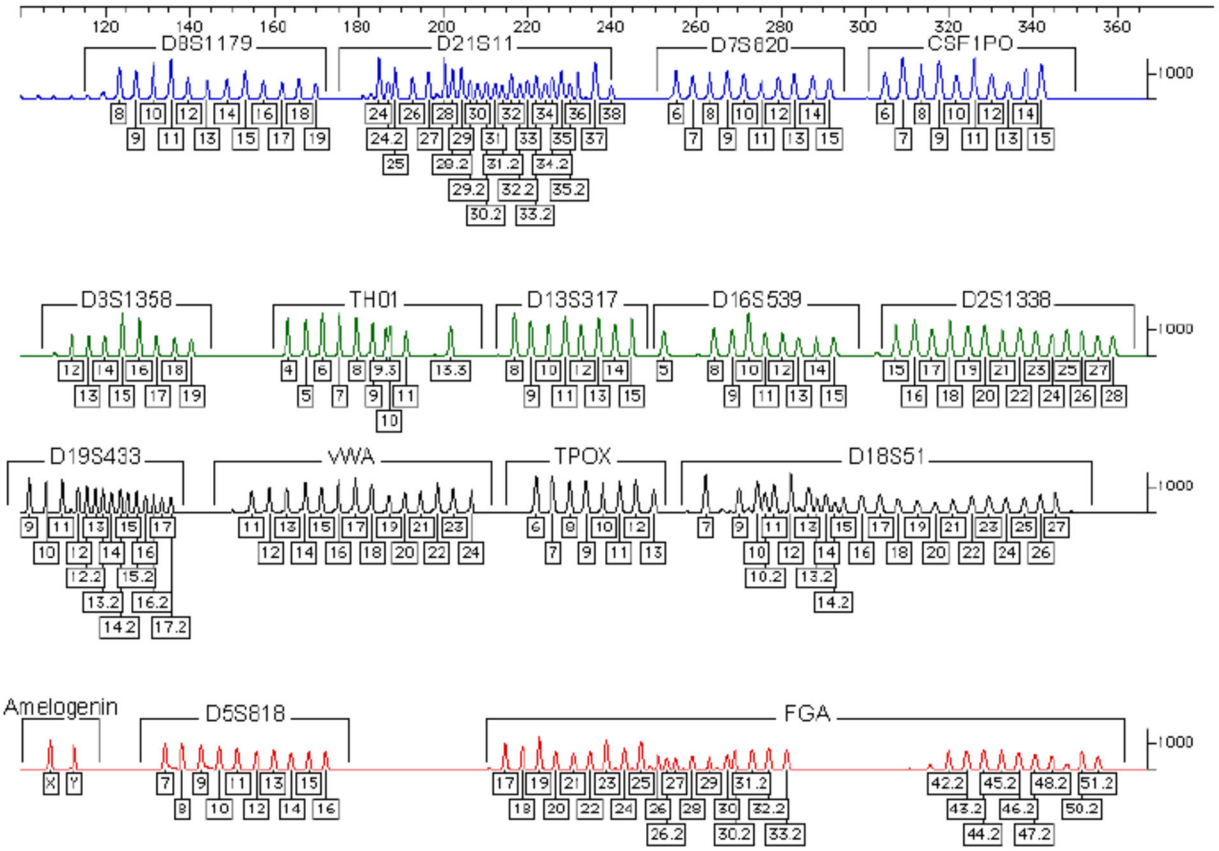
Assessment of Internal Lane Standard

The LIZ 500 internal size standard must have the expected size results for the 75.0, 100.0, 139.0, 150.0, 160.0, 200.0, 300.0, 340.0, 350.0, 400.0, and 450.0 base pair fragments. The ~250 fragment may vary in size. The peaks must be sizable but do not have to be above 100 RFU.



Assessment of Allelic Ladder

Examine the allelic ladders to determine that the software has assigned all allele designations correctly and the peaks are at least 100 RFU. The following alleles at the specific loci must be labeled.



Assessment of Controls

1. Amplification negatives and manipulation blanks should contain no profiles. When only a single spurious peak, peaks consistent with cross-talk, or known artifacts are present, no further troubleshooting is required and data analysis may be performed. Where peaks above the 100 RFU analytical threshold are present, bring to the TL. Potential courses of action include interpretation of a reduced number of loci or reporting the sample as inconclusive.
2. Amplification positives should contain the profile as given in the table below since these samples were previously reported by the MVRCL.

3. Amplification Positive Control Types:

Identifiler	Kit positive
D8S1179	13
D21S11	30
D7S820	10, 11
CSF1PO	10, 12
D3S1358	14, 15
TH01	8, 9.3
D13S317	11
D16S539	11,12
D2S1338	19, 23
D19S433	14, 15
vWA	17, 18
TPOX	8
D18S51	15, 19
Amelogenin	X
D5S818	11
FGA	23, 24

Identifiler Data Interpretation

AT	5s 100 RFU*
ST	5s 200 RFU*
PHR	High quality data ~60%
Major:Minor	Min. 45% difference
Minimum to interpret SS	Min. 1 but overall quality of data considered. RMP more common than 1 in 100 are reported as inconclusive.
Minimum to interpret SS major or 2 person	3 or more loci, examples in manual
Minimum to interpret 3,4,5 person	3 or more loci, examples in manual
Homozygote rule in mixtures	Distinct contrast between Major and Minor

* BCI will use 100 RFU for the threshold for MV data for all injection times. BCI requires use of the 5s injection time for basis of interpretation. Extended injection times can only be used to support assumptions (e.g., 3 alleles called in 5s with 1 allele just below threshold. Assumption of only 2 contributors and 4 alleles called in a 15s inject then can use the locus for stat.)

~~**30 Appendix B Miami Valley Regional Crime Lab (MVRCL) Legacy Data Procedures**~~

~~1. DNA Extracts~~

~~DNA extracts from the MVRCL may be tested by BCI. Refer to section 17 of the DNA Methods manual to begin testing procedures. The reagent blank associated with the MVRCL extract must be tested by BCI and meet the requirements set forth in the DNA QA manual section 9.5.1 and the DNA Methods manual in order to report additional STR kit results for MVRCL DNA extracts. Extracts and blanks will be entered into LIMS as items received. The chain of custody for the MVRCL DNA extracts and reagent blanks will be tracked in the case documentation.~~

~~2. Data Re-analysis~~

~~Data generated by the MVRCL may be re-analyzed using GeneMapper v1.4 software. For each amplification kit, refer to the DNA Methods manual unless addressed below. For MVRCL legacy data comparisons, random match probabilities (RMP) for single source and major single source profiles and restricted/unrestricted combined probability of inclusion (rCPI and CPI) for mixtures will be issued using PopStats.~~

~~The following situations are NOT considered a re-interpretation:~~

- ~~The generation of a letter for the comparison of 2 samples as a result of a CODIS high stringency match is not considered reinterpretation of legacy data.~~
- ~~If a casework (forensic) DNA profile interpretation has been previously documented regarding the genotypes that would be allowed for possible contributors, that interpretation is not considered re-interpretation.~~
- ~~The import of a MVRCL .fsa or .hid file into GeneMapper ID-X to compare only the allele calls to the CODIS entry is not considered re-interpretation, but an administrative check.~~

~~The following situations are considered re-interpretation:~~

- ~~Assessing/reevaluating allele calls and/or genotype calls (to include RFU values, potential allelic drop-out, number of contributors) for MVRCL data.~~
- ~~Changing the assumptions used for MVRCL data.~~
- ~~Adding allele/locus to an existing CODIS entry.~~
- ~~Removing alleles/loci from an existing CODIS entry.~~
- ~~Adding obligate allele indicators (+) to an existing CODIS entry.~~

~~Fusion Amplification Kit~~

~~Re-analyzing MVRCL Fusion with GeneMapper IDX software~~

1. ~~Review the MVRCL case information to determine which run contains the .hid files for the samples and controls for reanalysis. Import the sample, controls, and ladder files into GeneMapper IDX.~~
2. ~~Fusion analysis settings:~~

   ~~Analysis method: MV Fusion 6C 24s or 40s~~
   ~~Panel: PowerPlex_Fusion_6C_Panels_IDX_v1.2~~
   ~~Size Standard: WEN_ILS_500_CS~~
   ~~Bin Set: PowerPlex_Fusion_6C_Bins_IDX_v1.2~~
   ~~Stutter ratio: Marker specific (specific stutter values are loaded into the Panel manager)~~
   ~~Global Cut-off Value: 0.0 for all Marker Repeat Types~~

3. ~~The screenshots below demonstrate the correct settings within the Analysis method for 40s Fusion injections. The dye thresholds will be 100 RFU for the 24s injection time.~~
4. ~~Turn on the marker-specific stutter filters for N-2, N-3, N-4, N+3 and N+4.~~

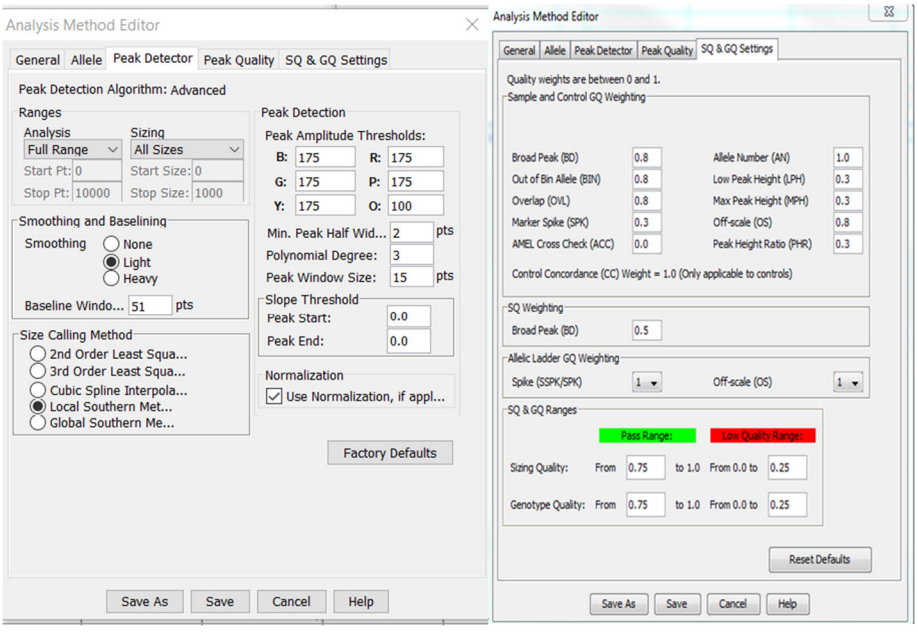
Fusion N-2, N-3, N-4, N-5 Stutter Filter % Values

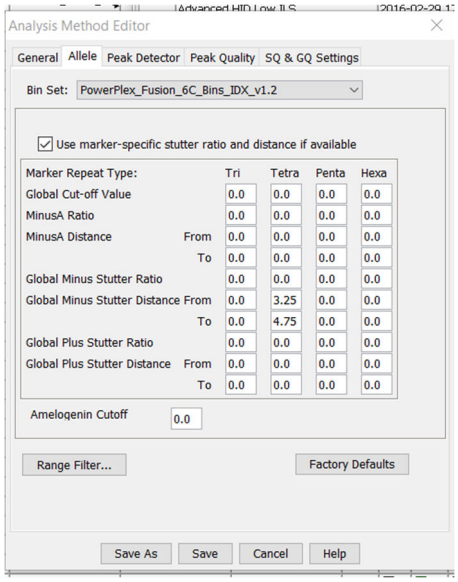
D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E
13.5	14.3/3.6 (N-2)	9	13	10.3	7.2
D16S1539	**D18S51**	**D2S1338**	**CSF1PO**	**Penta D**	
12	14.6	13.6	11.1	4.5	
TH01	**vWA**	**D21S11**	**D7S820**	**D5S818**	**TPOX**
4.8	14.4	12.7	9.7	11	5.4
D8S1179	**D12S391**	**D19S433**	**SE33**		
11.8	17.4	12.1/1.4 (N-2)	16.1/6.6 (N-2)		
D22S1045	**DYS391**	**FGA**	**DYS576**	**DYS570**	
16.8	9.4	12.4/1.2 (N-2)	12.5	13	

Fusion N+3, N+4, N+5 Stutter Filter % Values

D3S1358	D1S1656	D2S441	D10S1248	D13S317	Penta E
1.7	**2.3**	**1.8**	**1.3**	**2.2**	**1.9**
D16S1539	**D18S51**	**D2S1338**	**CSF1PO**	**Penta D**	
3	**2.8**	**2.2**	**3.7**	**3.7**	
TH01	**vWA**	**D21S11**	**D7S820**	**D5S818**	**TPOX**
1.5	**2.7**	**2.8**	**1.8**	**2.3**	**1.1**
D8S1179	**D12S391**	**D19S433**	**SE33**		
3.4	**2.7**	**2.6**	**3.3**		
D22S1045	**DYS391**	**FGA**	**DYS576**	**DYS570**	
9	**2**	**2.8**	**3.4**	**2.4**	

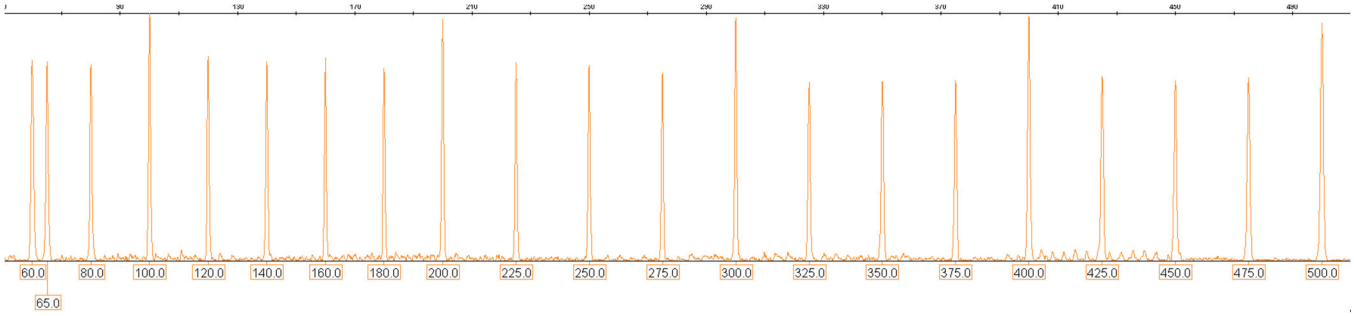
5. ~~The analysis range can be changed from "full range" to a more "partial range" to avoid the primer front and/or if sizing issues arise with the LIZ. Sizing needs to include the 60 bp peak as well as all of the other LIZ peaks through 500 bp to ensure correct sizing results.~~





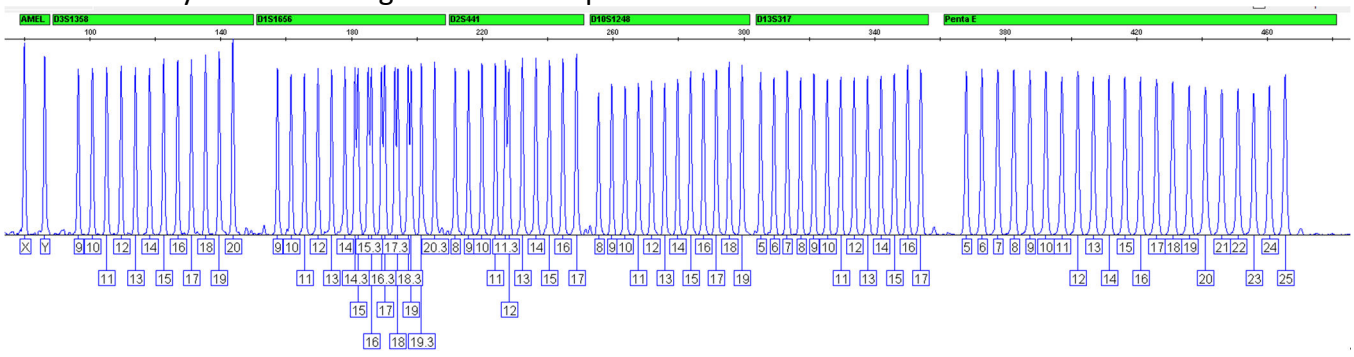
~~Assessment of Internal Lane Standard~~

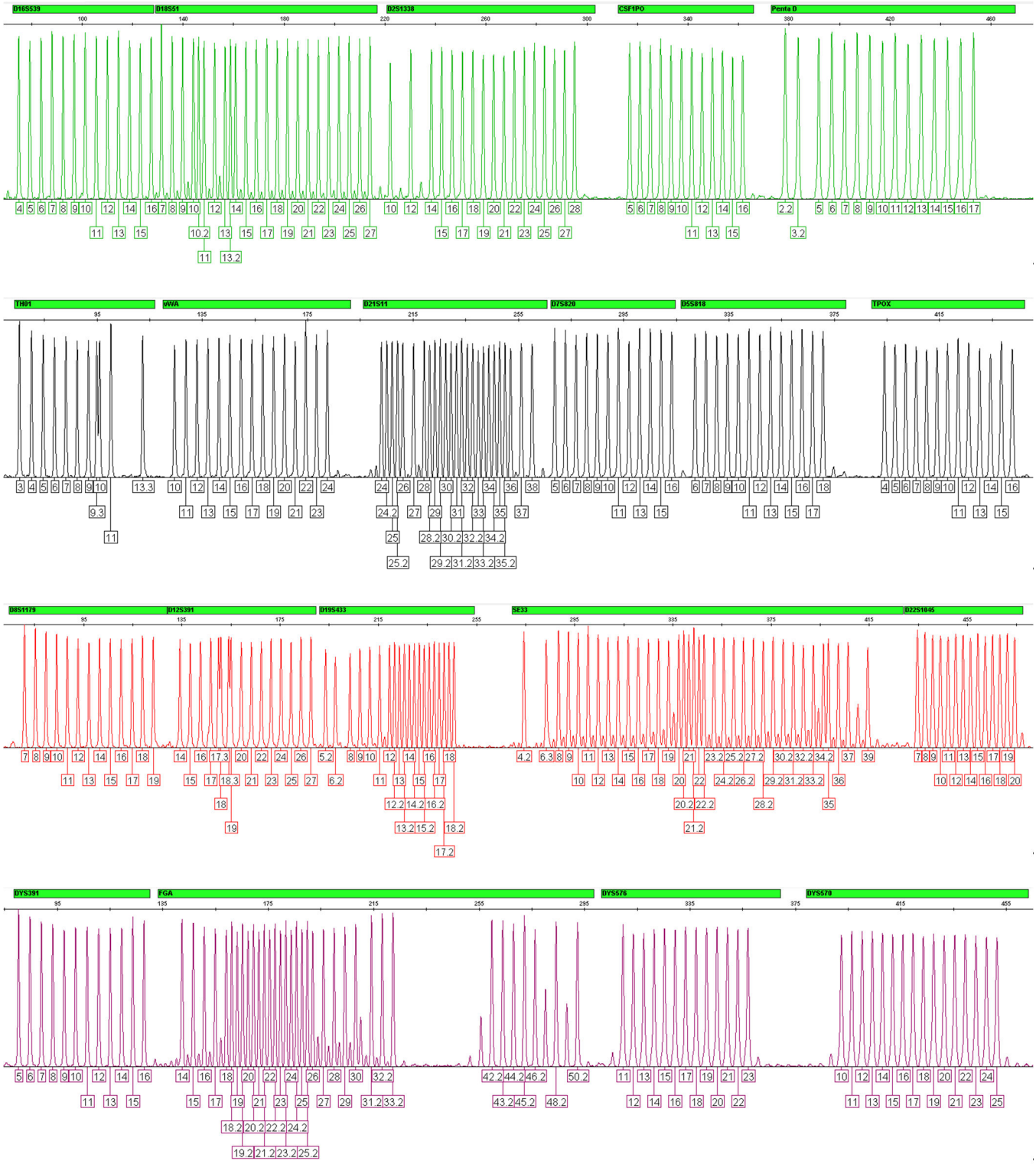
~~The WEN_ILS_500_CS internal size standard must have the expected size results for the 60.0, 65.0, 80.0, 100.0, 120.0, 140.0, 160.0, 180.0, 200.0, 225.0, 250.0, 275.0, 300.0, 325.0, 350.0, 375.0, 400.0, 425.0, 450.0, 475.0, and 500.0 base pair fragments. The peaks must be sizable but do not have to be above 100 RFU.~~



~~Assessment of Allelic Ladder~~

~~Examine the allelic ladders to determine that the software has assigned all allele designations correctly. The following alleles at the specific loci must be labeled and are at least 100 RFU.~~





~~Assessment of Controls~~

1. ~~Amplification negatives and manipulation blanks should contain no profiles. When only a single spurious peak, peaks consistent with cross talk, or known artifacts are present, no further troubleshooting is required and data analysis may be performed. Where peaks above the 100 RFU (24s injection) or 175 RFU (40s injection) analytical thresholds are present, bring~~

~~to the TL. Potential courses of action include interpretation of a reduced number of loci or reporting the sample as inconclusive.~~

2. ~~Amplification positives should contain the profile as given in the table below since these samples were previously reported by the MVRCL.~~
3. ~~Amplification Positive Control Types:~~

~~Fusion 6C~~	~~Kit Positive~~
~~Amel.~~	~~X, Y~~
~~D3S1358~~	~~17, 18~~
~~D1S1656~~	~~12, 13~~
~~D2S441~~	~~10, 14~~
~~D10S1248~~	~~13, 15~~
~~D13S317~~	~~9, 11~~
~~Penta E~~	~~7, 14~~
~~D16S1539~~	~~9, 13~~
~~D18S51~~	~~16, 18~~
~~D2S1338~~	~~22, 25~~
~~CSF1PO~~	~~12~~
~~Penta D~~	~~12, 13~~
~~TH01~~	~~6, 9.3~~
~~vWA~~	~~16, 19~~
~~D21S11~~	~~29, 31.2~~
~~D7S820~~	~~8, 11~~
~~D5S818~~	~~12~~
~~TPOX~~	~~11~~
~~D8S1179~~	~~14, 15~~
~~D12S391~~	~~18, 23~~
~~D19S433~~	~~13, 14~~
~~SE33~~	~~15, 16~~
~~D22S1045~~	~~16~~
~~DYS391~~	~~10~~
~~FGA~~	~~20, 23~~
~~DYS576l~~	~~18~~
~~DYS570~~	~~17~~

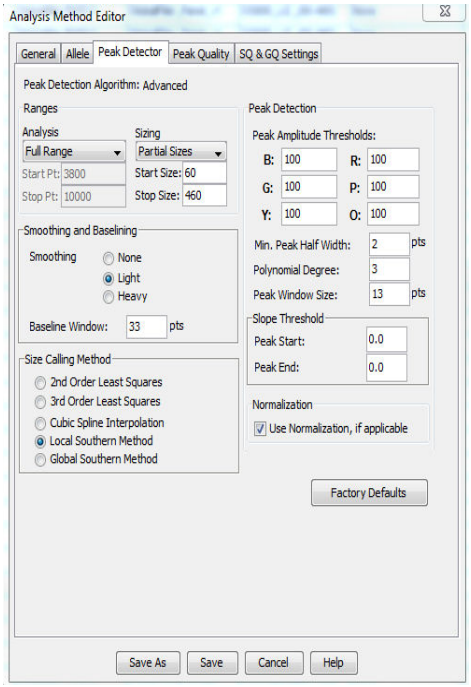
~~Fusion Data Interpretation~~

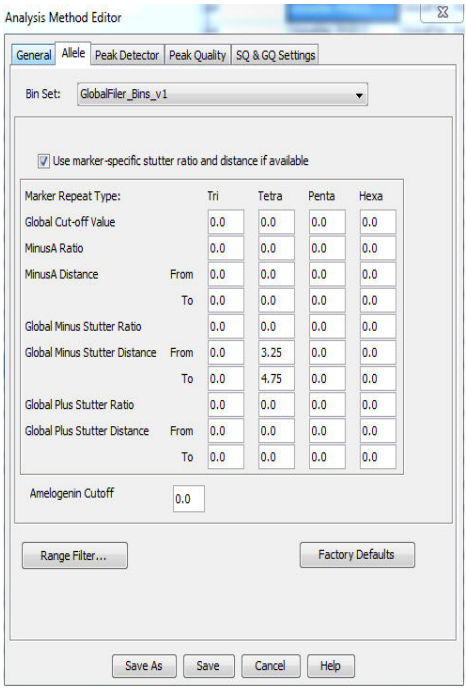
~~AT~~	~~24s 100 RFU, 40s 175 RFU~~
~~ST~~	~~24s 800 RFU, 40s 1100 RFU~~
~~PHR~~	~~High quality data ~60%~~
~~Major:Minor~~	~~Min. 40% difference~~
~~Minimum to interpret SS~~	~~Min. 1 but overall quality of data considered. RMP more common than 1 in 300 are reported as inconclusive.~~
~~Minimum to interpret SS maj~~	~~5 loci for 2 person; 10 loci for 3, 4 person~~
~~Minimum to interpret Mixed Major~~	~~5 loci for 2 person; 10 loci for 3, 4 person~~
~~Homozygote rule in mixtures~~	~~1200 RFU and ≥4x largest minor peak~~

~~**Globalfiler Amplification Kit**~~

~~**Re-analyzing MVRCL Globalfiler with Genemapper IDX software**~~

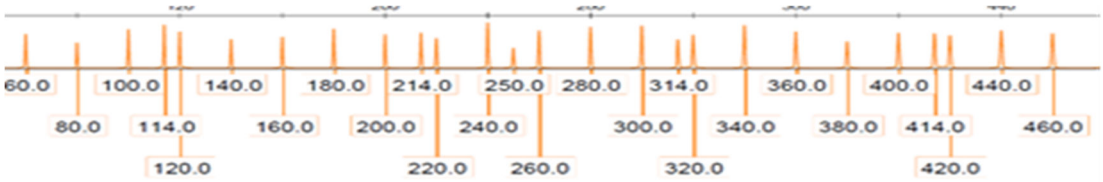
1. ~~Review the MVRCL case information to determine which run contains the .hid files for the samples and controls for reanalysis. Import the sample, controls, and ladder files into GeneMapper IDX.~~
2. ~~Globalfiler analysis settings:~~
   ~~Analysis method: Globalfiler 100 RFU~~
   ~~Panel: Globalfiler_Panel_v1-dup~~
   ~~Size Standard: GS600_LIZ_(60-460)~~
   ~~Bin Set: Globalfiler_Bins_v1~~
   ~~Stutter ratio: Marker specific (specific stutter values are loaded into the Panel manager)~~
   ~~Global Cut-off Value: 0.0 for all Marker Repeat Types~~
3. ~~The screenshots below demonstrate the correct settings within the Analysis method.~~
4. ~~Turn on the marker specific stutter filters for N-2, N-3, N-4, N+3 and N+4.~~
5. ~~The analysis range can be changed from "full range" to a more "partial range" to avoid the primer front and/or if sizing issues arise with the GS600 LIZ. The first allele bin is located after the 60 bp peak and is located at D2S441. Sizing needs to include the 60 bp peak as well as all of the other LIZ peaks through 460 bp to ensure correct sizing results.~~





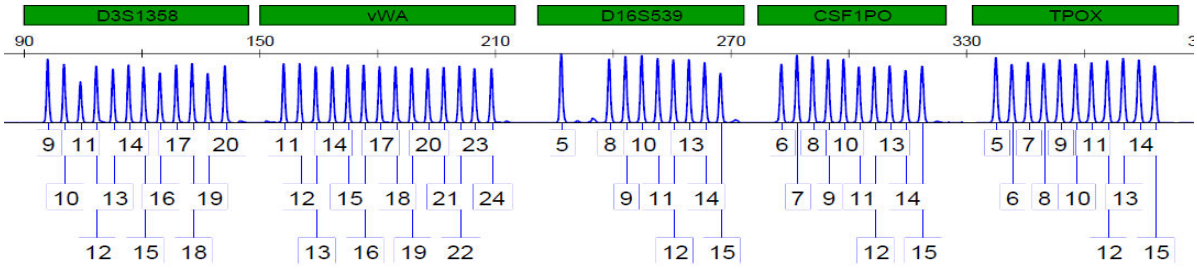
~~Assessment of Internal Lane Standard~~

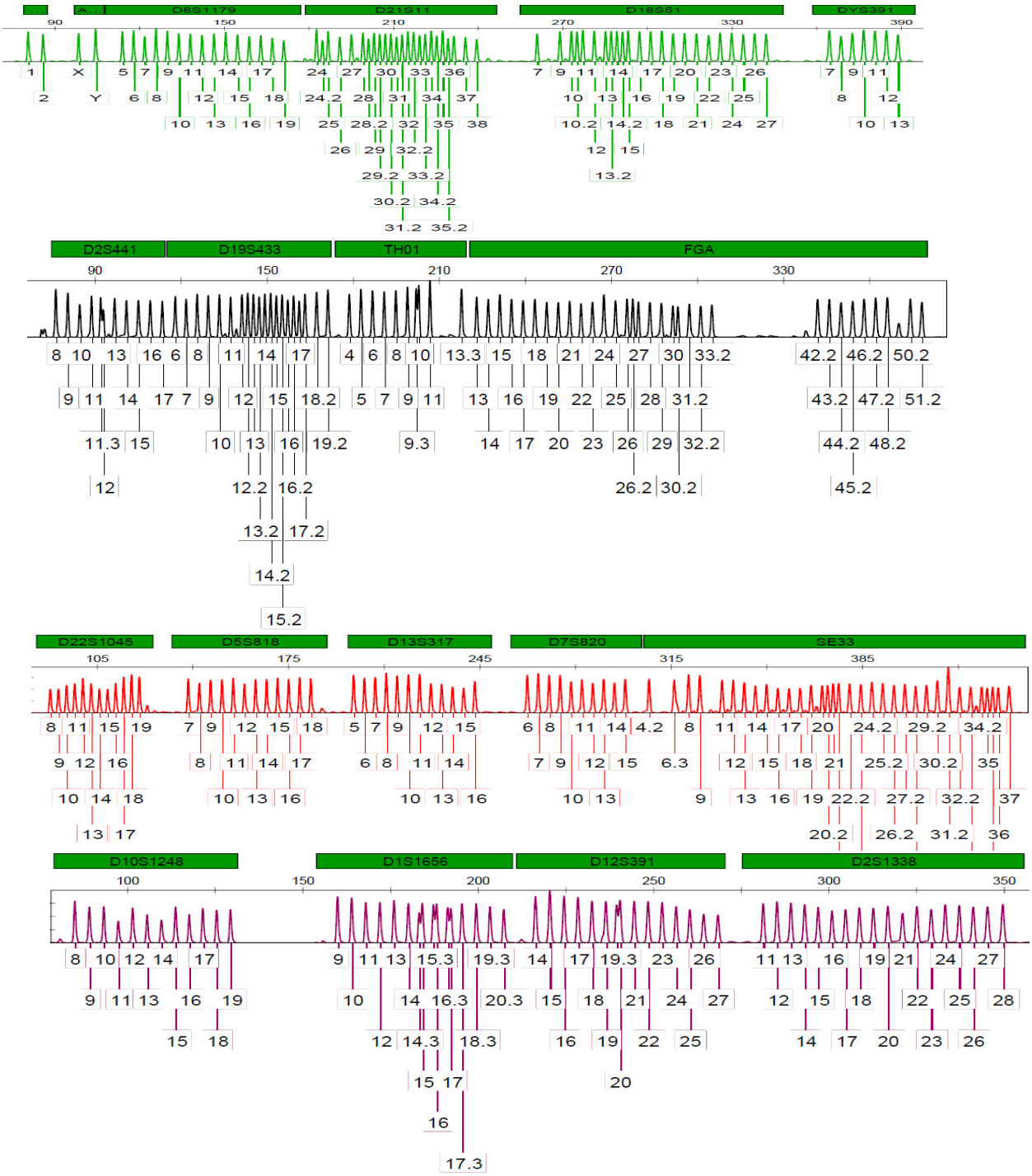
~~The LIZ 600 internal size standard must have the expected size results for the 60.0, 80.0, 100.0, 114.0, 120.0, 140.0, 160.0, 180.0, 200.0, 214.0, 220.0, 240.0, 250.0, 260.0, 280.0, 300.0, 314.0, 320.0, 340.0, 360.0, 380.0, 400.0, 414.0, 420.0, 440.0, and 460.0 base pair fragments. The peaks must be sizable but do not have to be above 100 RFU.~~



~~Assessment of Allelic Ladder~~

~~Examine the allelic ladders to determine that the software has assigned all allele designations correctly. The following alleles at the specific loci must be labeled and are at least 100 RFU.~~





~~**Assessment of Controls**~~

1. ~~Amplification negatives and manipulation blanks should contain no profiles. When only a single spurious peak, peaks consistent with cross-talk, or known artifacts are present, no further troubleshooting is required and data analysis may be performed. Where peaks above the 100 RFU analytical threshold are present, bring to the TL. Potential courses of action include interpretation of a reduced number of loci or reporting the sample as inconclusive.~~
2. ~~Amplification positives should contain the profile as given in the table below since these samples were previously reported by the MVRCL.~~

3. Amplification Positive Control Types:

Globalfiler	Kit Positive 007
D3S1358	15, 16
vWA	14, 16
D16S1539	9, 10
CSF1PO	11, 12
TPOX	8
Y-Indel	2
Amel.	X, Y
D8S1179	12, 13
D21S11	28, 31
D18S51	12, 15
DYS391	11
D2S441	14, 15
D19S433	14, 15
TH01	7, 9.3
FGA	24, 26
D22S1045	11, 16
D5S818	11
D13S317	11
D7S820	7, 12
SE33	17, 25.2
D10S1248	12, 15
D1S1656	13, 16
D12S391	18, 19
D2S1338	20, 23

Globalfiler Data Interpretation

AT	100 RFU
ST	200 RFU
PHR	High quality data ~65%
Major:Minor	Min. 40% difference
Minimum to interpret SS	Min. 1 but overall quality of data considered. RMP more common than 1 in 300 are reported as inconclusive.
Minimum to interpret SS maj or 2 person	5 loci for 2 person; 10 loci for 3, 4 person
Minimum to interpret 3,4,5 person	5 loci for 2 person; 10 loci for 3, 4 person
Homozygote rule in mixtures	1200 RFU and ≥4x largest minor peak

Identifiler Amplification Kit

Re-analyzing MVRCL Identifiler with Genemapper IDX software

1. Review the MVRCL case information to determine which run contains the .hid files for the samples and controls for reanalysis. Import the sample, controls, and ladder files into GeneMapper IDX.

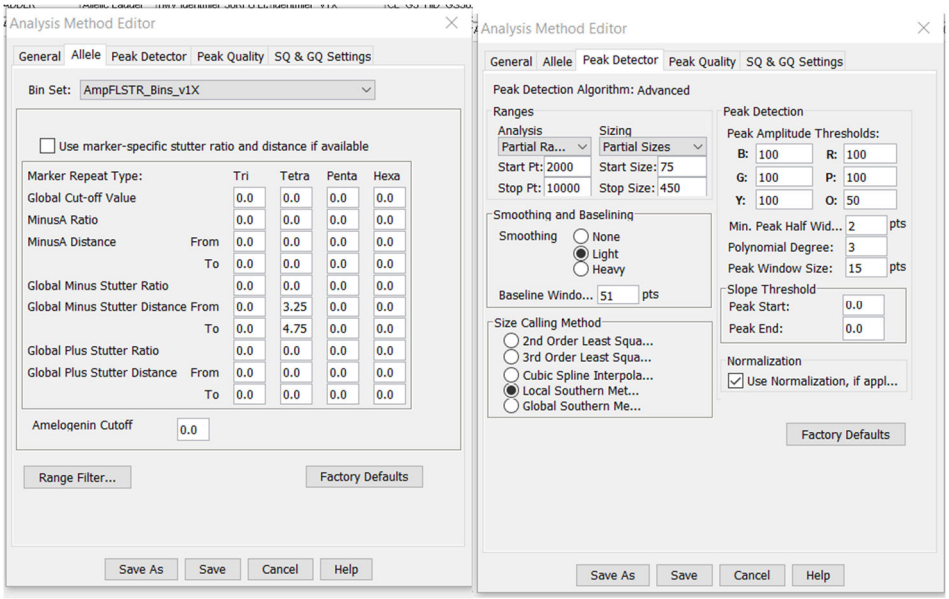
~~2. Identifiler analysis settings:~~

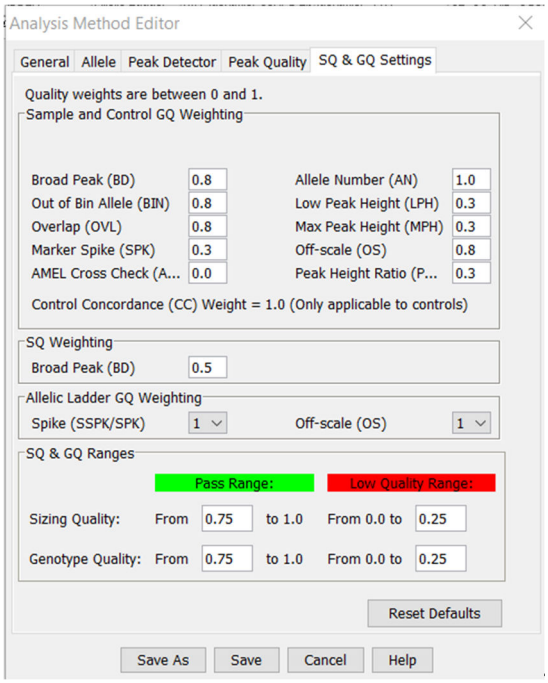
~~Analysis method: MV Identifiler 100 RFU~~
~~Panel: Identifiler_v1X~~
~~Size Standard: CE_G5_HID_GS500~~
~~Bin Set: AmpFLSTR_Bins_v1x~~
~~Stutter ratio: Do not use marker specific (specific stutter values are loaded into the Panel manager)~~
~~Global Cut-off Value: 0.0 for all Marker Repeat Types~~

~~3. The screenshots below demonstrate the correct settings within the Analysis method.~~

~~4. Turn off the marker-specific stutter filters.~~

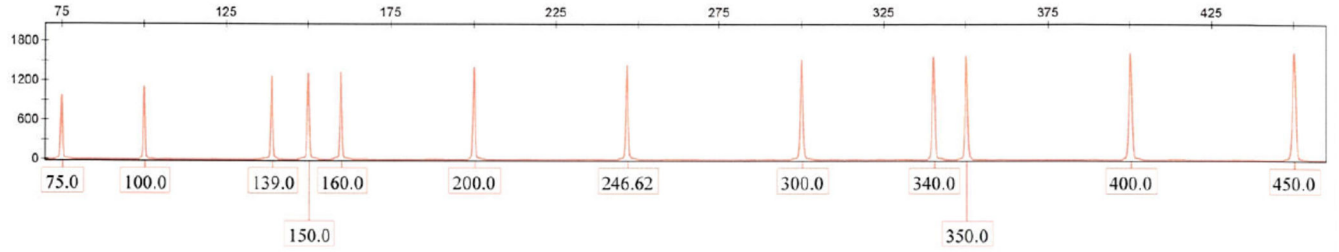
~~5. The analysis range can be changed from "full range" to a more "partial range" to avoid the primer front and/or if sizing issues arise with the LIZ. Sizing needs to include the 75 bp peak as well as all of the other LIZ peaks through 450 bp to ensure correct sizing results.~~





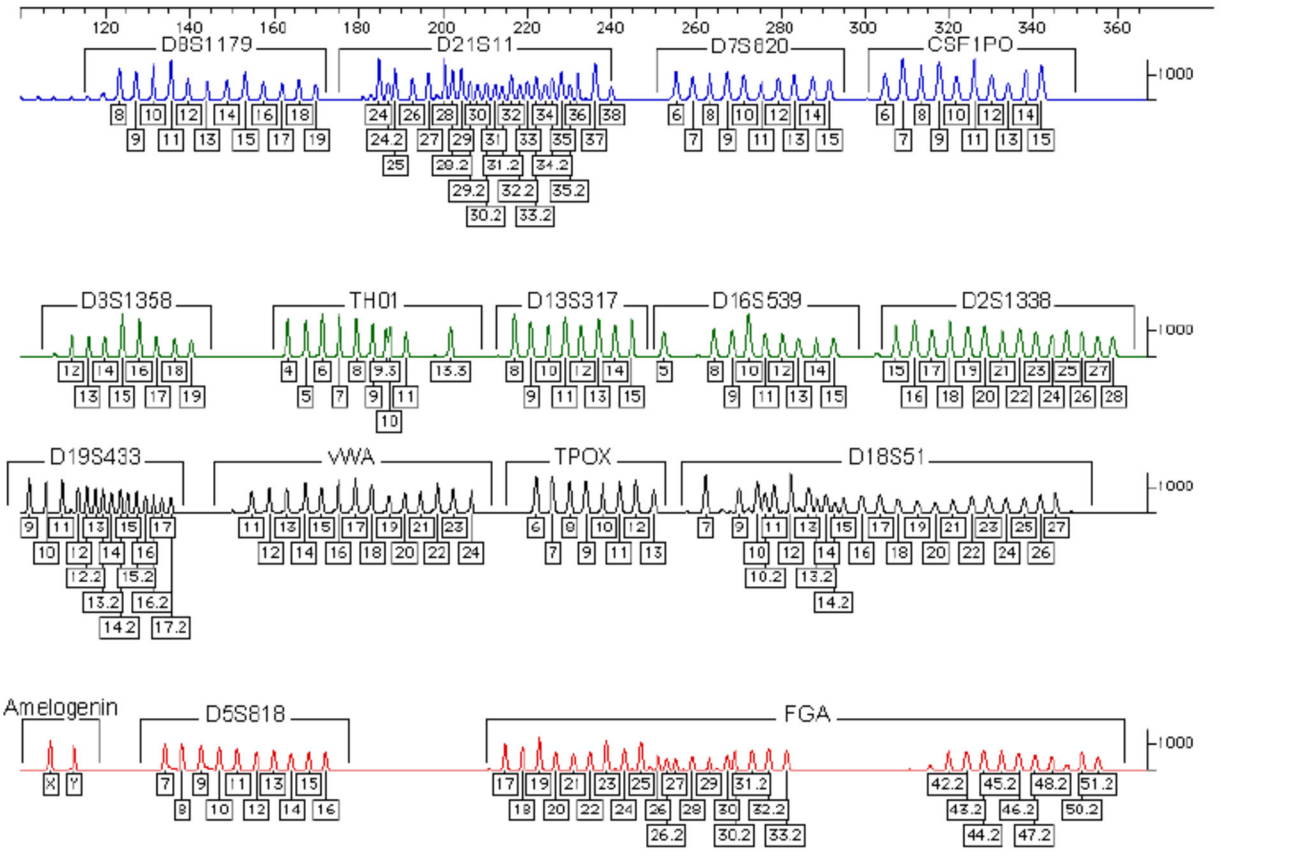
~~Assessment of Internal Lane Standard~~

~~The LIZ 500 internal size standard must have the expected size results for the 75.0, 100.0, 139.0, 150.0, 160.0, 200.0, 300.0, 340.0, 350.0, 400.0, and 450.0 base pair fragments. The ~250 fragment may vary in size. The peaks must be sizable but do not have to be above 100 RFU.~~



~~Assessment of Allelic Ladder~~

~~Examine the allelic ladders to determine that the software has assigned all allele designations correctly and the peaks are at least 100 RFU. The following alleles at the specific loci must be labeled.~~



~~Assessment of Controls~~

1. ~~Amplification negatives and manipulation blanks should contain no profiles. When only a single spurious peak, peaks consistent with cross-talk, or known artifacts are present, no further troubleshooting is required and data analysis may be performed. Where peaks above~~

~~the 100 RFU analytical threshold are present, bring to the TL. Potential courses of action include interpretation of a reduced number of loci or reporting the sample as inconclusive.~~

2. ~~Amplification positives should contain the profile as given in the table below since these samples were previously reported by the MVRCL.~~
3. ~~Amplification Positive Control Types:~~

~~Identifiler~~	~~Kit positive~~
D8S1179	13
D21S11	30
D7S820	10, 11
CSF1PO	10, 12
D3S1358	14, 15
TH01	8, 9.3
D13S317	11
D16S539	11,12
D2S1338	19, 23
D19S433	14, 15
vWA	17, 18
TPOX	8
D18S51	15, 19
Amelogenin	X
D5S818	11
FGA	23, 24

~~Identifiler Data Interpretation~~

~~AT~~	~~5s 100 RFU*~~
~~ST~~	~~5s 200 RFU*~~
~~PHR~~	~~High quality data ~60%~~
~~Major:Minor~~	~~Min. 45% difference~~
~~Minimum to interpret SS~~	~~Min. 1 but overall quality of data considered. RMP more common than 1 in 100 are reported as inconclusive.~~
~~Minimum to interpret SS major or 2 person~~	~~3 or more loci, examples in manual~~
~~Minimum to interpret 3,4,5 person~~	~~3 or more loci, examples in manual~~
~~Homozygote rule in mixtures~~	~~Distinct contrast between Major and Minor~~

~~* BCI will use 100 RFU for the threshold for MV data for all injection times. BCI requires use of the 5s injection time for basis of interpretation. Extended injection times can only be used to support assumptions (e.g., 3 alleles called in 5s with 1 allele just below threshold. Assumption of only 2 contributors and 4 alleles called in a 15s inject then can use the locus for stat.)~~

3. MVRCL Comparison Report Examples

Example 1 for MVRCL Reporting – Data run at MVRCL but not previously reported

To:	BCI/Competency Testing Abby Schwaderer, QA Administrator BCI Laboratory Division Quality Assurance Program London, OH 43140	BCI Laboratory Number: Analysis Date: Date testing started at MV	23-12345 Issue Date: March 14, 2023
		Agency Case Number:	20-01234
Offense:	Rape	MVRCL Case Number:	20-04321
Subject(s):	Morningstar		
Victim(s):	Decker		

<u>Submitted on March 02, 2023 by Lewis Maddox to the Miami Valley Regional Crime Laboratory:</u> (GET THIS FROM MVRCL PAPERWORK IF NOT PREVIOUSLY REPORTED BY MVRCL)

MV # Parent Item
MV # Parent Item
MV # Parent Item
MV # Parent Item

Data generated at the Miami Valley Regional Crime Laboratory were analyzed and interpreted in the London laboratory based on BCI interpretation protocols.

Item	DNA Conclusions
MV001a. Bedsheet	DNA profile consistent with Decker – the estimated frequency of occurrence of the DNA profile is rarer than 1 in 1 trillion□ unrelated individuals. Morningstar – excluded
MV001b. Underwear	Mixture (2 contributors) Consistent with contributions from: • Decker – expected contributor • Unknown male – sufficient for comparison Morningstar – excluded
MV001c. Decker MV001d. Morningstar	Profile used for comparison purposes

Remarks

The eligible DNA profiles (Items MV001b and MV001d) have been entered into the CODIS database in accordance with state and national regulations, where regular searches will be performed. If investigative information becomes available or a profile is removed from CODIS, your agency will be notified.

Additional DNA comparisons can be made upon submission of a reference standard consisting of two oral swabs.

<u>Analytical Detail</u>

Samples from MVRCL 20-04321 were not available for retesting by BCI. DNA profiling was performed at the MVRCL using PCR with the Fusion STR kit on samples MV001a, MV001b, MV001c and MV001d. The data were analyzed and reported above.

Example 2 for MVRCL Reporting – Data run at MVRCL and previously reported, additional comparisons or BCI interpretation reported

			Amended
To:	Police Department	BCI Laboratory Number:	18-81236
	123 Main Street	Analysis Date:	Issue Date:
	Any Town, OH 12345	When BCI started	April 17, 2018
Offense:	Rape	Agency Case Number:	2013-1235
		MVRCL Case Number	21-001234
Subject(s):	Frank Thompson, Sam Smith		
Victim(s):	Jane Doe		

Submitted on November 03, 2022 by John Smith:

1. One manila envelope containing known standard from Frank Thompson

The standard listed above was tested and compared with data provided by the Miami Valley Regional Crime Laboratory. The data were re-analyzed and interpreted in the London laboratory based on BCI interpretation protocols.

Items	DNA Conclusions
External vaginal swabs collected from Jane Doe (MVRCL Case 21-001234 001a)	Mixture (2 major contributors) Major - Consistent with contributions from: • Jane Doe – expected contributor • Frank Thompson – The estimated proportion of the population that cannot be excluded as possible major contributors to the mixture of DNA profiles in the sperm fraction is 1 in 300,000□ unrelated individuals. The remainder of the mixture contains DNA that is not of sufficient quality for comparison to a standard from any individual.
1 DNA Standard – Frank Thompson	Profile used for comparison purposes
□ Based on the national database provided by the National Institute of Standards and Technology	

Remarks

An eligible DNA profile has been entered by the MVRCL into the CODIS database in accordance with state and national regulations, where regular searches will be performed. If investigative information becomes available or a profile is removed from CODIS, your agency will be notified.

Additional sample from BCI item 1 is available should independent analysis be requested. All remaining items submitted to BCI will be returned to the submitting agency. The remaining DNA extract from BCI item 1 will be retained by the laboratory.

This report supplements the original testing performed on MVRCL Case X Item Y.

Analytical Detail

DNA profiling was performed using PCR with the Globalfiler® STR kit on a sample from Item 1 and compared to the reanalyzed sample from Item 001a (see the MVRCL case [#] report dated xxx).

Example 3 for MVRCL Reporting – DNA extract from MVRCL typed and reported with BCI interpretation

NOT AMENDED IN THIS SITUATION

To:	Police Department	BCI Laboratory Number:	18-81236
	123 Main Street	Analysis Date:	Issue Date:
	Any Town, OH 12345	April 15, 2018	April 17, 2018
Offense:	Rape	Agency Case Number:	2013-1235
		MVRCL Case Number	21-001234
Subject(s):	Frank Thompson, Sam Smith		
Victim(s):	Jane Doe		

Submitted on November 03, 2022 by John Smith:

1. One manila envelope containing known standard from Frank Thompson
2. One manila envelope containing DNA extract from the external vaginal swabs collected from Jane Doe (MVRCL Case 21-001234 001a)
3. One manila envelope containing extraction reagent control XXXXXX associated with MVRCL Case 21-001234 001a

Items	DNA Conclusions
1 DNA standard – Frank Thompson	Profile used for comparison purposes
2 External vaginal swabs DNA extract from Jane Doe (MVRCL Case 21-001234 001a)	Mixture (2 major contributors) Major - Consistent with contributions from: • Jane Doe – expected contributor • Frank Thompson – The estimated proportion of the population that cannot be excluded as possible major contributors to the mixture of DNA profiles in the sperm fraction is 1 in 300,000□ unrelated individuals. The remainder of the mixture contains DNA that is not of sufficient quality for comparison to a standard from any individual.
3 Extraction reagent control	No DNA profile
□ Based on the national database provided by the National Institute of Standards and Technology	

Remarks

An eligible DNA profile has been entered by the MVRCL into the CODIS database in accordance with state and national regulations, where regular searches will be performed. If investigative information becomes available or a profile is removed from CODIS, your agency will be notified.

AS APPROPRIATE Additional sample from each item is available should independent analysis be requested. All remaining items submitted to BCI will be returned to the submitting agency. The remaining DNA extracts will be retained by the laboratory.

Analytical Detail

The DNA extracts from Items 2 and 3 were generated at the MVRCL and transported to the London Laboratory for additional testing. DNA profiling in the London laboratory was performed using PCR with the Globalfiler® STR kit on a sample from Item 1 and previously extracted samples for Items 2 and 3.

Additional Report Wording:

A DNA profile developed by the MVRCL (Item MV X) has been removed from the CODIS database. The removal of this DNA profile does not imply any previous DNA reports issued by MVRCL are incorrect or invalid.

The MVRCL CODIS entry was based on DNA profiling previously performed at the MVRCL using PCR with the Minifiler® STR kit. The DNA profile developed by MVRCL (Item MV X) doesn't meet BCI's current interpretation guidelines and has been removed from the CODIS database. The removal of this DNA profile does not imply any previous DNA reports issued by MVRCL are incorrect or invalid.

DNA profiling was previously performed at the MVRCL using PCR with the Identifiler® and Minifiler™ STR kits on a sample from Item MV X. The Identifiler® data were reanalyzed and reported above.

DNA profiling was previously performed at the MVRCL using PCR with the Globalfiler® STR kit on a sample from Item MV X. The data were reanalyzed and reported above.