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Forensic Biology Casework Protocol Manual – DNA Screening Supplemental

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Section 1 Scope of the DNA Screening Supplemental

This document is designed to provide an alternative, expedited workflow process and report writing method for the screening of sexual assault evidence in cases with female victims and male alleged assailants. Protocols for specific laboratory procedures are located in the current version of the Forensic Biology Casework Procedures Manual (FBCP). Using this modified workflow, analysts qualified in biological screening as well as DNA analysis through quantitation will use biological screening techniques to identify samples of potential interest. These samples will then be extracted and quantified. Analysts will then use the total human DNA and male DNA quantification results to identify the extracts which are best suited for amplification and analysis. These results will be issued in a biological screening report. Extracts will either be dried down for long-term storage or analyzed by a qualified DNA analyst.

Section 2 Abbreviations

RBS	Reagent blank sperm
RBSS	Reagent blank sperm + substrate
RBE	Reagent blank epithelial
RBsub	Reagent Blank substrate
RBQ	Reagent blank questioned (direct)
No Male	No male DNA detected
Low Male	Female:male ratio is 10:1 or higher. Y-STRs recommended.
Low DNA	Quantitation indicates that consumption of sample is recommended.
STR	Quantitation indicates that the extract is suitable to proceed to STR testing.

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Section 3: Selection, sample size and extraction

Analysts rely upon available information to make assessments about which items are most appropriate for analysis on a case-by-case basis. The extraction process for a single sample may accommodate up to two full swabs (or four half-swabs) per tube. Elution volumes of 40 uL are routinely used for all questioned extracts, as sample size permits. When a sample is used in its entirety to create an extract:

- Both the bench notes and the sample (noted in red on tube) must clearly indicate that the sample has been consumed.
- Half the extract must be retained unless written permission for consumption of the sample has been obtained.

Semen-containing items (differential extraction):

- Analysts follow the two flowcharts in the appendices of the FBCP to determine which samples are appropriate for differential extraction using the QIAcube automated wash protocol.
- If an item has been consumed for analysis, the substrate must be extracted, either combined with the sperm pellet or worked separately:
 - Items likely to have very high amounts of epithelial cells, such as vaginal swabs, cervical swabs, rectal swabs, or underwear cuttings from crotch area should be processed without combining the sperm pellet with the substrate.
 - Items likely to have relatively lower amounts of epithelial DNA, such as external genital swabs, condom swabs, and cuttings from clothing other than underwear, may have the substrate added to the sperm pellet for a single extract.
- Slides are made from all sperm pellets, but they do not need to be stained or read prior to issuing the screening report. Slides are repackaged with original evidence.

Non-semen-containing items (direct extraction):

- Analysts use case-specific information to choose stains testing positive to a presumptive test for blood, breast swabs, and other miscellaneous swabs most likely to have probative value, based on case scenario.
- Hairs are not routinely extracted in the first round of testing.

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Reference samples:

- Reference samples are cut into tubes, but not extracted during the screening process.
- Reference sample cutting tubes may be stored with extracts created from the same kit if STR analysis is pending, or repackaged with the original evidence if no further analysis is pending.

Reagent blanks:

- Reagent blanks are created for each set of extracted samples taken through the same extraction protocol (e.g. sperm/substrate, epithelial and/or direct) on the same day by the same analyst.
- Reagent blanks are named by extraction type, date and analyst (RBS 14-1025CD).
- Since reference samples are not extracted at this stage, it is not necessary to create a reagent blank for the reference samples.

Documentation:

Items which are sampled must include the following information documented in the bench notes:

- Extraction date (or cut date if not extracted)
- Approximate amount of evidence sampled
- EZ1-XL instrument used
- QIAcube instrument used
- Elution volume

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Section 4: Quantitation

- Extracts should ideally be quantitated within seven days of the start of the extraction process.
- More than one batch of extracts may be included on the same quantitation plate. In such cases, the plate name should include the initials of each analyst, and each analyst is responsible for documentation of their own samples.
- Plates are named according to the format QD14-1030CD/MLC. This indicates kit used, date, and initials of plate analyst(s).
- If the total volume of the consumed-sample extract is 40 μ L, only one quantitation reaction will be used in order to conserve extract. All other extracts should be quantitated with replicate reactions.
- Quantitation is performed and standard curves assessed according to the protocol in FBCP. Female:male DNA ratios are calculated by subtracting male DNA concentration from total DNA concentration, then dividing by male DNA concentration.
- Quantitation results can be used to assess whether or not an extract is likely to be single-source, either entirely or effectively for the purposes of STR amplification:
 - Single-source female: Intimate samples from a female's evidence collection kit (that is, body swaps or underwear from an evidence kit) with no male DNA detected are likely to be single source. Alternatively, samples with detectable male DNA present in a ratio of greater than 10:1 female:male are likely to appear as single-source samples upon amplification, since any minor component present is very unlikely to be suitable for comparison.
 - Single-source male: Samples where the male DNA quantitation value is higher than the total human quantitation value are likely to only yield results suitable for comparison for the male contributor(s).
 - NOTE: either of the above situations relies upon the assumption that a sample is likely to have DNA from only one contributor of the gender in question. Some case scenarios (e.g. multiple possible assailants or the possible presence of a consent partner as well as an assailant) preclude this assumption.
- Quantitation results are used as a screening tool to determine suitability for future testing. A sample decision tree for this process is shown in Appendix B. The guidelines below apply to typical samples from female victim evidence kits:

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- Extracts with no male DNA detected will be retained with no further testing. (Exceptions would include condom samples where a female profile may have probative value.)
- Extracts with any detected male quantitation value but undetermined total human DNA are best suited for STR analysis only if the entire remaining evidence / extract can be consumed.
- Extracts likely to contain single-source male DNA, where both human and male DNA are detected by quantitation, are suitable for STR analysis.
- Extracts with male DNA present at ratios greater than 10:1 female:male are not suitable for STR analysis, but may be suitable for Y-STR analysis.
- Extracts with male DNA present in a ratio of 10:1 female:male or less, but with a total human DNA concentration less than 0.05 ng/uL, are best suited for STR analysis only if the entire remaining extract is consumed.
- Consumed samples: Extracts likely to contain mixtures are ready for STR DNA analysis when ALL three conditions listed below are met. If total human DNA or male DNA concentration is below the minimum concentrations, the extract is best suited for STR analysis only if the entire remaining extract is consumed.
 - male DNA is present in a ratio of 10:1 female:male or less
 - total human DNA concentration equal to or greater than 0.10 ng/uL
 - male DNA concentration is equal to or greater than 0.01 ng/uL
- Not-consumed samples: Extracts likely to contain mixtures are ready for STR DNA analysis when ALL three conditions are met. If total human DNA or male DNA concentration is below the minimum concentrations, the extract is best suited for STR analysis only if the remaining sample is extracted and all the combined extract from the sample is consumed.
 - male DNA is present in a ratio of 10:1 female:male or less
 - total human DNA concentration equal to or greater than 0.05 ng/uL
 - male DNA concentration is equal to or greater than 0.005 ng/uL
- Following quantitation, all extracts for which STR DNA analysis is not pending must be dried down using DNASTable LD (see FBCP for procedure). Extracts pending analysis may be stored in an extract refrigerator (for less than one week) or extract freezer.
- Copies of standards and controls documentation, as well as quantitation documentation, are included in the bench notes for each case file.

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Section 5 Report writing

The guidelines for Biological Screening reports, as listed in FBCP, also apply to DNA Screening reports. DNA Screening reports contain additional conclusions based on quantitation results. The following tables contain samples of results, conclusion and opinions appropriate for reporting various case results. These are not all inclusive and may be modified slightly on a case by case basis.

For samples not amplified based on Quantifiler Duo results	Report
No male DNA detected	Quantification results do not indicate the presence of male DNA. No further analysis was performed on this sample.
Female: Male ratio >10:1	<p>Quantification results do not indicate the presence of sufficient male DNA for STR analysis. No further analysis was performed on this sample.</p> <p>This sample may be suitable for Y-STR analysis. For more information, please contact the laboratory's DNA Technical Manager, XXXX XXXX (269-XXXX) or XXX.XXX@alaska.gov</p>
<p>Male DNA detected but human DNA quantity is undetermined</p> <p>Consumed sample - Not (effective) single-source Male DNA detected below 0.01 ng/uL OR human DNA concentration < 0.1 ng/uL</p> <p>Non-consumed sample - Not (effective) single-source Male DNA detected below 0.005 ng/uL OR human DNA concentration < 0.05 ng/uL</p>	<p>Quantification results indicate the presence of male DNA. Based on the low quantity of DNA present in this sample, the recommended amplification procedure would consume the remaining sample in its entirety.</p> <p>No further analysis will be performed on this sample without written permission for consumption of the sample in its entirety. For more information, please contact the laboratory's DNA Technical Manager, XXXX XXXX (269-XXXX) or XXX.XXX@alaska.gov</p>

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All others	Report
Samples suitable for STR analysis based on Quantifiler Duo results	Quantification results indicate the presence of male DNA. This sample is suitable for further analysis.

Section 6 Completion of DNA Screening

- Upon completion of DNA screening, evidence is transferred from the DNA screening analyst to a DNA analyst if further analysis is pending or to evidence storage if no further analysis is pending.
- Extracts and un-extracted reference sample cuttings which are destined for DNA analysis are all stored in a designated freezer until DNA STR analysis is completed for the case. After the case is completed, the DNA analyst is responsible for either discarding or archiving remaining extracts, as appropriate.

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DNA Screening Review Checklist

Date: _____	Analyst: Tech. Review: Admin. Review:
Technical Review Started: _____	
Administrative Review Started: _____	
Lab Number: _____	

Pages are numbered correctly, lab case #, item # and analyst initials are on each page			
Requesting agency, agency case #, lab case #, and officer's name are correct			
Item numbers / packaging / descriptions on report / notes are consistent with RLS/LIMS			
The type of examination (visual, stereoscopic, ALS) and testing performed is documented in notes			
Item descriptions are consistent with clothing/evidence images present (if applicable)			
The location of all chemical testing performed is documented in the notes (if applicable)			
All isolated stains/samples are documented and numbered correctly (if applicable)			
Evidence consumed to create extract is noted as such in bench notes			
Verification reviews conducted are documented in the notes (if applicable)			
The location and disposition of all trace evidence is documented			
Worksheets contain all lot #s and expiration dates for all reagents used			
Q-PCR plate set-up is documented			
Q-PCR standard curve printouts: Results are acceptable.			
Q-PCR Initial Template Quantity is documented by 7500 printout.			
Retained items created in LIMS; all retained and unexamined items HELD in LIMS			
The "FUTURE TECH" flag has been tripped for the case, if applicable (samples suitable for Y-STR)			

Check grammar/spelling/punctuation in report			
Results/conclusions/opinions are given for each item tested			
Conclusions/opinions drawn from results comply with laboratory guidelines			
Conclusions/opinions drawn from results are supported by documentation in the notes			
Known samples requested, if appropriate			
Report signed in LIMS			
All case related notes and attached/scanned documents are present			
Technical reviewer is in review history for each page of the bench notes			
SOPs are linked to request in LIMS			
Chain of Custody for all tested items can be tracked through RLS and LIMS			
Assign DNA holding to DNA Supervisor			

VERIFICATION REVIEW	Analyst:	Date:
Semen/ABAcad® p30 Test		
Species/ABAcad® HemaTrace®		
Hairs - Stereoscopic		

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Appendix A: Revision History

Since no prior versions of this document exist, there is no revision history.

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Appendix B: *Sample Decision Tree - assumes male contributor is probative, and only one likely male contributor*

