DOCUMENT STRUCTURE

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Evidence Definitions

Forensic FBI QAS 7.3.1 / Database FBI QAS Standard 7.3.1

Batch: collection of cases being worked simultaneously by an analyst.

Evidence: Per the Quality Assurance Manual, items received at the laboratory with an agency case number and item number are considered to be evidence; and the CODIS individual characteristic database items are treated as reference materials. Extracts that are being retained (as required in the Forensic Biology Procedure Manual) become evidence once they are dried and packaged for long-term storage. If the parent item (from which the extract was created) is being returned to the submitting agency, the extract will be created as its own item of evidence in the LIMS.

Known (or K) sample: a reference sample collected directly from an identified individual, typically collected as a buccal swab or drawn blood.

Questioned (or Q) sample: evidence collected from an alleged crime scene, for which the source(s) of any DNA present on the evidence may be unknown.

Set: collection of samples within a particular laboratory process in LIMS-DNA being worked by an analyst.

- **Closed Set**: this set is being closed due to administrative error and is not used in analysis. Gets a note about why it is closed, and reviewer must "approve" it. Will be recorded in your LIMS-DNA Appendix and is retained in the LIMS-DNA database and is part of your final discovery.
- **Submitted Set**: this set is being submitted for review or is in the process of being reviewed; sets must be submitted to proceed with the next process set; this occurs after you hit the complete button on the Completion page.
- **Approved Set**: this is a set that has been tech reviewed and signed off/finalized and is part of your final discovery; no longer visible on the dashboard (use Lookup function to open); requires a supervisor to unlock for editing.

Unique identifiers:

Casework: To ensure that each sample can be distinguished throughout processing, tubes are labeled with laboratory case number, item number, sub-item number, and/or differential fraction, as applicable.

Database: Database samples are typically batch-processed. Each batch of samples is assigned a batch name comprised of the date of initial digest or batch set-up, the processing analyst's initials,

and a plate identifier to indicates processing order (such as 23-0101LDS_A, 23-0101LDS_B, and so on). Racks of sample tubes comprising a batch are labeled with the batch identifier. To ensure that each sample can be distinguished throughout processing, sample tubes within the batch are assigned codes that include plate identifier and order within the plate (e.g., A12). In combination with a batch name, this creates a unique identifier for each database sample.

Work product: a material that is generated as a function of analysis, which may include extracts during analysis, spermatozoa search slides/extract slides, and amplified products.

Retaining Technical Records and Casework DNA Extracts

AR 3125 7.5.1.1

Documentation Generated During DNA Analysis

Observations of casework and database analysis must be documented contemporaneously with the lab work process.

Casework technical records consist of:

- Bench notes, created and retained in the LIMS case file, include evidence images
- Electropherograms, mixture interpretation worksheet, statistics pages, and specimen detail reports (from CODIS), created outside of LIMS and retained in the LIMS case file
- Central log documentation, created in LIMS-plus DNA and archived in SharePoint
- Raw CE files are stored together in a zipped folder in SharePoint; quantification raw data files and quantification supplemental workbook are uploaded to SharePoint individually.

Database technical records consist of:

- Central log documentation, stored in SharePoint
- Raw CE files are stored together in a zipped folder in SharePoint
- Exported GeneMapper ID-X database projects, stored in SharePoint

Forensic Biology Casework Bench Notes

Bench notes consist of any documentation generated during analysis of a case and specific to that case. All bench notes pages in the case record contain the case number, analyst's initials and item # (if appropriate, i.e., digital images).

Note: For Proficiency Tests, the results pages of the paperwork from the test provider are completed and become the first pages of the bench notes.

Bench notes generated outside of LIMS (electropherograms and statistics pages) are placed in the case images for the request in the LIMS. All bench notes must be in LIMS, and the report must be marked draft complete (by the analyst) before submitting the case for technical review. Upon completion of the technical and administrative reviews, only the final bench notes are retained in the LIMS. See the PDF guidance document on the network for more details on preparing bench notes for LIMS retention.

The LIMS worksheets contain details of all the items processed and include the item packaging, contents and description, images of the evidence processed (when applicable), documentation of all presumptive tests performed and the test results, the location of all testing, the location of all isolated stains/samples, trace evidence collected (if applicable), the reagents used, and the date testing began (where applicable).

In addition to the LIMS worksheets, the bench notes for each case may contain the following:

- Electropherograms for all the samples amplified. All electropherograms must contain the lab case # and item #.
    - If the sample has no labeled peaks, the electropherogram must also include the primer peaks [Analysis method: 3500 Blank-Casework]
    - When an OL is a true allele or a potential tri-allele exists, a casework artifact view is also required that includes the relevant locus for an allelic ladder and the sample with the potential tri-allele.
    - The following handwritten notations may be included on the electropherogram:
        - Artifacts (i.e., pull-up, dye blobs, etc.) are struck and initialed (or electronic equivalent) on the full view electropherogram and the artifact view
        - For peaks in stutter positions (that appear close to the expected stutter percentages) indicate the % of the peak relative to the main peak and the maximum expected stutter percentage (for example, 12%>5% or 5.2%~5%). When the peak can be reasonably interpreted as elevated stutter, add a notation to this effect. When it is not possible to discern whether the peak is a stutter peak or a true allele, add "A/TA".
        - If able to determine a major vs. minor profile, ( ) around the minor alleles. [ ] is used to indicate alleles that could not be separated into major/minor components.
        - 'NS' or 'NS major' (and reason for non-inclusion) at loci determined not to be suitable for inclusion in a statistical analysis.
- Optional: Mixture Interpretation Worksheet is used to document profile deduction. Documentation included on this worksheet includes:
    - Stated assumptions
    - Reference profile used
    - Reasoning for deduction (or not deduced)
    - Profile for CODIS entry if applicable
- Popstats/YHRD printouts for all samples for which a statistical analysis was performed will include:
    - Specimen ID: lab case # and Item#
    - Comments section: add any additional info (ie sperm fraction, major profile)
    - Print for all reported populations

A review checklist may be used to facilitate the review, but it is not maintained. The milestones in LIMS are used to document that the elements of review (as in the Forensic Biology Procedure Manual) have been completed.

Forensic Biology Casework Central Log Records

LIMS-plus DNA is the module used during analysis to capture all the elements of the lab process from extraction through electrophoresis. Batch controls, reagents and instrumentation used are also captured in the LIMS-plus DNA module.

A review checklist may be used to facilitate the review of the batches within LIMS-plus DNA, but it is not maintained. The milestones in the module are used to document that the elements of review (as in the Forensic Biology Procedure Manual) have been completed.

Upon completion of the technical review of a batch, the central log documentation is archived in SharePoint.

It is recognized that not all cases may be submitted to technical review simultaneously. However, all relevant reagent blank(s) should be confirmed at a minimum before any cases are submitted for technical review.

Forensic Biology Database Central Log Records

Each batch of database samples assigned to a DNA analyst will be named with DB followed by the batch date (typically, this is the day you take custody of the evidence or first begin analysis) and the analyst's initials, often followed by a letter which indicates the batch's order for that analyst's annual workload. (i.e.YY-MMDDinitials DB24-0102MLC_A). Upon completion of the technical review of a batch, the central log documentation is retained in SharePoint.

The templates for the worksheets that comprise the central log are controlled documents and can be found in SharePoint. The templates may be modified as required for the batch. The central log for each batch of cases will contain at least the following information in an appropriate format:

A central log review checklist, completed and initialed by the analyst and technical reviewer. Analyst will complete the first column of boxes before handing in for technical review

An SDIS Import Reconciliation Report

- This captures the number of new specimens uploaded to SDIS, as well as any specimen updates or upload problems.

A batch worksheet includes a batch identifier and interpretation dates. For each item in the batch:

- Sample number (LIMS case number)
- Sample code
- Specimen category
- Gender listed in LIMS
- Amplification/genetic analyzer plate well number
- Quantification and amplification set-up, if applicable
- Concordance check results, if applicable

- Suitability for upload
- If rejected, reason for rejection and course of follow-up action
- Comments about unusual samples (such as off-ladders, tri-alleles, etc.)
- Documentation of technical review including reviewer initials

The Standards, Controls and Reagents worksheet(s) detailing:

- Batch identifier
- Analysis methods and dates
- Specific instrument(s) used
- Master mix volumes
- Raw data file names
- Reagent lot numbers and reagent expiration dates. (reagents not used can be deleted from worksheet)

If quantification is performed, the Quantification Set-up Worksheet, to include the following:

- Plate layout
- Reagent lot numbers and reagent expiration dates
- Master mix calculations

If quantification is performed, the Quantification Experiment Results Report, to include the following:

- Experiment summary
- Note quantification instrument used (add to front page)
- Note passive reference passing (add to front page)
- Note date that interpretation/decisions were completed (add to front page)
- Plate layout
- T-L, T-S, and T-Y standard curves
- Results table

If electrophoresis results indicate that a sample should be re-extracted, re-amplified or re-injected, the reason should be documented in the comments/notes field of the batch worksheet, such as poor injection or (partial) drop-out.

Retention of DNA extracts

- All questioned extracts created during analysis will be retained.
- If an entire item of evidence (e.g., penile swabs, fingernail scrapings, contact DNA swabs, etc.) is used for DNA extraction owing to potentially limited amounts of biological material, then at least half of the DNA extracted from that item will be retained, unless written permission from Department of Law for consumption of the sample has been obtained and documented in LIMS.
- Best practice for if a casework reference sample extract is generated but no corresponding questioned samples are proceeding to amplification, the reference sample extract can EITHER be amplified with documentation included with the bench notes or dried down and stored

with the evidence. If the extract is not amplified, the analyst must ensure that corresponding ICS and reagent blank controls are either amplified or also dried down for future analysis at the same time as the reference sample extract. If controls will need to be amplified along with the reference extract, that must be clearly documented on the reference extract packaging.

- In situations where the entire item of evidence has been used for DNA extraction and when quantitation results suggest that the entire volume of the DNA extract will be required for PCR amplification to attempt to obtain interpretable data, the laboratory shall require written permission from the Department of Law to consume the entire extract.
- Database (CODIS) samples are considered as reference material. As such, they are not treated as evidence, and extracts are not retained.

Retention of electronic data

- All raw data files generated during casework and database electrophoretic analysis shall be retained in SharePoint.
- Because discipline manuals contain sufficient information to recreate the GeneMapper ID-X project from the raw data files, casework GeneMapper ID-X project files are not retained long term and may be deleted after the technical and administrative reviews of a batch are completed. Database projects are retained because there is no individual case record for each profile.

Rejected data in technical records

The LIMS-DNA work instructions provide instructions for capturing the required information when data is rejected.

- Rejected quantification data
  - If an entire quantification run is rejected, the run is still captured in the LIMS-DNA paperwork. A comment should be made in the notes section to indicate the reason the data was rejected, and the data was not used.
  - When an individual quantification result is rejected, a reason must be noted in the notes section of the original quantification set.
- Rejected electrophoresis data
  - When an entire injection (or run) is rejected, the raw data file is retained in SharePoint. The relevant detection set in LIMS-DNA must give a reason why the data was rejected (e.g., no passing allelic ladder, no data obtained from injection, master mix did not include ILS, etc.). The raw data file should be listed in the associated LIMS-DNA detection set; corresponding IDX project can be "data not used" if applicable
  - When individual sample data is rejected, the relevant detection set in LIMS-DNA or the relevant electropherogram(s) must give a reason why the data was rejected (e.g., broad peaks detected, injection failed, etc.).
- Rejected LIMS generated bench notes
  - Corrections to custom forms related to sample processing and results can be made by modifying the data in the relevant field(s). Amendments are tracked via the LIMS audit trail and no additional documentation is required.
  - When images embedded within LIMS-generated bench notes need to be updated, both the original and final are retained as attachments to the evidence item.
- Rejected .pdfs of files generated outside of LIMS
  - When the technical review process indicates that new pages must be added to address corrections (recalculation of population frequency statistics) or additions (re-amplification of a sample), the original pages must be kept in the bench notes. A note must be added to the rejected data indicating why the data was rejected, along with analyst date and initials, and clear marking to indicate that the data was not used. New pages must clearly indicate what was added, as well as analyst date and initials.
  - If an individual calculation or observation is rejected, it can be struck and corrected with the analyst date and initials noted. If the reason for rejection was incorrect or missing calculation, the corrected number is considered documentation of the reason for rejection. Other reasons for rejection must be stated in the bench notes.
- Rejected observations (i.e. digital images)

- Duplicate images do not need to be retained when multiple images were taken of an observation with the intent of retaining the best quality image in the technical record.
- Images that do not reflect an original observation of evidence need not be retained (ex. accidental image of analyst's foot, blurry images)

Forensic Biology Discipline Locker Key Policy

Forensic FBI QAS Standard 7.2 / Database FBI QAS Standard 7.2

Evidence lockers in the Forensic Biology discipline are self-assigned. An analyst may choose any locker(s) for storing evidence. When lockers are not in use, keys are stored in the locks.

When a locker is being claimed by an analyst for long-term use, the analyst shall take custody of the key in the LIMS.

A master set of locker keys is stored in the locked key box in the biology open office area. Access to the key box is restricted to laboratory management.

If one of the locker keys is lost, the discipline supervisor shall be notified.

Forensic Biology Literature Review Policy

Forensic FBI QAS Standard 16.1.2 / Database FBI QAS Standard 16.1.2

All qualified analysts in the Forensic Biology discipline are expected to present an article of scientific literature for members of the discipline to read, as appropriate to their areas of competency. Literature reviews are included during the quarterly technical meetings.

Analysts document their literature reading in SharePoint in the meeting agenda. In addition to the check boxes for attendance, for each meeting where literature was reviewed, the meeting minutes will have a list where analysts will record the dates by which they completed the assigned reading.

Articles reviewed by the group are retained in Biology Literature in Sharepoint with appropriate tags completed (such as year of review, year of publication, and topic) so that they can be found by search. At least some of the annual literature reviews should be current (published within the last 1-2 years).

Literature review documentation is monitored annually by the DNA Technical Manager.

Extended Absence Retraining Policy

Forensic FBI QAS standard 6.12 and 6.12.1 / Database FBI QAS Standard 6.10 and 6.10.1

When an analyst is away from the laboratory for an extended period (three months or longer, or one in which a scheduled proficiency test is missed), the technical leader shall evaluate the need for retraining, assess the extent of any necessary retraining, and approve the retraining plan. At a minimum, the returning analyst must complete and pass an internal competency test before resuming casework analysis. The scope of the competency test and authorization to resume casework are the responsibility

Forensic Biology Evidence Retention Policy

Forensic FBI QAS Standard 7.4 and 7.4.1 / Database FBI QAS Standard 7.4 and 7.4.1

Items submitted for Forensic Biology examinations are routinely triaged for processing, with the items most likely to yield relevant, interpretable results being given priority. Additional testing will not typically occur once probative results are obtained. More details on item selection policies are provided on the crime lab webpage.

The following guidelines will apply to most cases. Evidence may be returned or sent to another laboratory at the request of the submitting agency.

- Sexual assault kits will be retained by the laboratory indefinitely.
    - When creating sub-items from a kit, the kit evidence type must be cleared/removed from the sub-item.
- Stains/samples isolated (by laboratory personnel) from larger items will be retained indefinitely
- Non-consumed questioned DNA extracts will be retained
- Stains/samples isolated by law enforcement (or laboratory staff at the crime scene) and submitted as swabs for analysis will be retained by the laboratory if they are tested by the DNA unit.
- Untested questioned samples/items may be returned to the submitting agency after completion of the case
- Control swabs/stains are not typically processed and may be returned
- Reference samples are typically retained indefinitely (including database reference samples as well as casework)
    - These items may be returned if no other items are being retained in a case.

Sample/Evidence consumption policies for forensic casework and database analysis are found in the Forensic Biology Procedure Manual.

Annual Forensic Biology Quality Review, Monitoring Validity of Results, Performance Monitoring, and Annual Case Review

ISO 17025 7.7, AR 3125 7.7.6, Forensic FBI QAS Standard 3.3 and 3.4 / Database FBI QAS Standard 3.3 and 3.4

Annual Forensic Biology Quality Review

In fulfillment of FBI QAS standard 3.3, an annual review of the quality system in the Forensic Biology discipline will occur concurrently with the lab-wide annual quality system review.

The quality review of the Forensic Biology discipline will be completed under the direction and approval of the DNA Technical Manager. At a minimum, the quality review of the Forensic Biology discipline will include the following:

- Audit (internal and/or external) of the Forensic Biology discipline
- Summary of AR 7.7.6 performance monitoring for Forensic Biology discipline members
- Review of a sample of case files (annual case review), in fulfillment of FBI QAS 3.4. The scope of this review will be modified annually and approved by the Technical Manager, in advance of the review. At a minimum it must include a representative sample of cases worked, specifically including cases which demonstrate the elements outlined later in this section.
- Inventory of long-term biological evidence storage
- Inventory of location in biology that are not intended for long-term storage of evidence
- Inventory of GMID-X and CODIS staff elimination databases
- Collection of feedback from discipline members regarding improvements to discipline manuals
- Review and updates to manuals
- Review of all verifications, validations, and performance checks. Follow-up as required.
- Review of CODIS operations documentation
- Conduct an overview of all Forensic Biology CARs and QARs for the preceding year.
- Conduct an overview of all Forensic Biology DRFs for the preceding year.
- Assessment of discipline-wide adherence to literature review policy
- Assessment of discipline-wide adherence to laboratory and QAS continuing education requirements

Documentation of the Forensic Biology quality system annual review will be by memo to the lab QA Manager.

Monitoring validity of results (ISO 17025 7.7.1)

The laboratory has procedures for monitoring the validity of results.

Area	Procedure	Monitoring plan	Review documentation
Use of appropriate controls at all stages of casework and database analysis, including both positive (reference material) and negative controls [ISO 7.7.1a]	Procedures for use and review of control data are found in FBPM	100% of control samples run with casework or database undergo technical review. Failing controls are documented through either a Quality Assurance Review or a Contamination Assessment Form. These forms of quality documentation are reviewed quarterly to identify trends.	Casework control documentation is retained in LIMS-DNA; other records are retained in SharePoint
Maintenance and performance checks for critical equipment (includes functional checks and intermediate checks on balances) [ISO 7.7.1c and e]	Maintenance and performance check procedures and plans are found in the FBGLM	At least annually, hard copies of these records are scanned to SharePoint. At the time of scanning, they are reviewed or completeness and correctness. Problems (such as missed maintenance) are addressed through Quality Assurance Reviews, which are in turn are reviewed quarterly to identify trends.	Records are retained in SharePoint
Review of ongoing changes in reagent chemistry performance	Reagent verification of new reagent lots is performed using procedures found in the FBGLM.	The DNA Technical Manager will maintain spread sheets of key quality metrics to identify trends in kit performance as soon as possible.	Spreadsheets are maintained in SharePoint.
Review of CODIS operations	Procedures for CODIS maintenance are found in the COD.	A CODIS Administrator creates a monitoring plan annually.	Records are retained in SharePoint
Review of reported results [ISO 7.7.1i]	The annual case review procedure includes both casework and database and is based in part on	The DNA Technical Manager defines the scope and plan for the annual case review.	Records are stored in SharePoint.

	existing technical review procedures found in FBPM; any modifications to those procedures are specified in the case review plan.		
Newly qualified database and casework analysts have required mentoring sessions with a senior analyst at critical decision points, including sampling/triage and amplification. This is typically done for the first three batches following completion of training.	The procedure for the mentoring program is described in the FBTM.	The Training Coordinator facilitates the mentoring and ensures that the mentoring analyst either takes hand-off cases or does the reviews for the mentored analyst.	Casework mentoring is documented with a case activity (QA-Monitoring activity); database mentoring is documented with a note on the review checklist. Review of effectiveness of the mentoring is performed by interview with the Training Coordinator after each mentored batch.

Performance Monitoring (AR 3125 7.7.6)

Each year, the DNA Technical Manager will design a performance monitoring plan to fulfill the requirements of OSAC Forensic Biology ASB 123 as well as AR 7.7.6:

- The laboratory's monitoring of performance by comparison with results of other laboratories shall, where available and appropriate for the laboratory activities, demonstrate successful performance in at least one proficiency test or an approved alternative means of interlaboratory comparison for each accredited discipline per calendar year at each location (AR 7.7.2.1.b).
- The laboratory shall monitor the performance of all personnel who perform laboratory activities. The monitoring shall demonstrate successful performance in at least one proficiency test, other interlaboratory comparison, or intralaboratory comparison per calendar year in each accredited discipline in which the individual is authorized to conduct work. In the event that the preceding options are not available or appropriate, observation-based performance monitoring is acceptable. (AR 7.7.4)
- The performance monitoring plan ensures inclusion of a portion of the components/parameters and equipment/technologies within each accredited discipline.
- Forensic Biology Scope of Accreditation includes:

Discipline: Biology		
Component/Parameter	**Item**	**Key Equipment/Technology**
DNA Profile Determination	Short Tandem Repeat (STR) Y-Short Tandem Repeat (Y-STR)	Capillary Electrophoresis
Individual Characteristic Database	DNA Profile	National DNA Index System (NDIS)
Physical Comparison	DNA Profile	Software Program
Qualitative Determination	Body Fluid	Capillary Electrophoresis Chemical Fluorescence Spectroscopy General Microscopy Immunoassay

The performance monitoring plan addresses the elements which fall under these components, key equipment and technologies, as well as summarizing risk factors associated with each, and how performance monitoring is assessed using a combination of external and internal tests.

The process for performance monitoring shall…	**By…**
ensure that results are not known or readily available to the participant being monitored	Using external test providers, or a test preparer (typically the DNA Technical Manager and/or the DNA Training Supervisor) who keeps test results in an undisclosed location during the testing period
ensure use of approved methods by the individual(s) whose performance is being monitored	Using the technical review process or requiring sufficient documentation with the test to check for use of correct method. NDIS eligibility test ensures correct method through its test design.
establish criteria for successful performance prior to the monitoring activity being conducted	Defining passing criteria for external tests in appropriate manuals, and clearly stating passing criteria on in-house test directions.
require a mechanism to ensure the quality of the monitoring activity prior to personnel performance being monitored	Ensuring that external test providers meet AR 7.7.7 criteria. Extra copies of in-house tests are created and taken prior to test distribution to analysts.
require notification to ANAB within 30 days when the expected result is not attained during any monitoring activity	Notifying the Quality Assurance Manager promptly of non-conforming test results.
Retain the following records for performance monitoring: a) discipline(s) monitored; b) design of the monitoring activity; c) expected results; d) location, when more than one location is associated with a single accreditation certificate;	Archiving the records in the LIMS and/or SharePoint

e) records submitted to a proficiency test provider, if applicable; f) appropriate technical records; g) evaluation of results and action taken for unexpected results; and h) feedback on individual performance provided to the participant.	

Assessment of performance monitoring:

External proficiency tests

- Performance of analyst lab techniques including blood and semen presumptive tests, extraction, quantification, amplification, and capillary electrophoresis is monitored semiannually by ground truth proficiency tests created and graded by vendor providers.
- Passing criteria are full concordance with expected vendor results, with exceptions made for differences resulting from lab-specific policies that are clearly documented.
- Proficiency test results are monitored by lab QA Manager, DNA Technical Manager, and CODIS administrator as soon as results are available.

Practical performance monitoring tests

- Performance of screening and sampling techniques not addressed by proficiency tests will be monitored by administering a set of ground truth mock casework evidence for processing. These samples will be extracted, quantified, and amplified (if necessary) to assess analyst success at the given task.
- Passing criteria are test-specific but generally require accurate results as compared to ground truth, consistent results between participants, correct DNA profiles, and no introduced contamination, as applicable
- Specific answer keys are required for all in-house prepared tests and include the range of acceptable answers, including when results must be positive or negative as well as when inconclusive may be appropriate, if applicable

Mixture interpretation tests

- In accordance with OSAC forensic biology ASB 123, performance of mixture interpretation protocols will be monitored by administering a set of ground truth mixtures to a subset of about half of the interpreting analysts per year for interpretation. This test is designed to ensure that the full range of STR and Y-STR interpretation is addressed through a combination of external and internal testing.
- Passing criteria will be identified in advance for each mixture. Generally speaking, passing criteria will require that analysts perform and document their interpretation with high

consistency (no more than three minor discrepancies per analyst per mixture), and that the conclusion is not incorrect with respect to the ground truth (if inconclusive results are appropriate, this will be specified in the test answer key).

Annual NDIS Eligibility Test

- Casework analysts qualified to upload forensic profiles into CODIS are required to take the annual review of CODIS eligibility administered by NDIS.
- Passing criteria and evaluation are determined by NDIS.

NOTE: Observation / witnessing of relevant casework is a tool for mitigating risk, not performance monitoring)

- Performance elements for some laboratory techniques are not suitable for mock casework/database ground truth samples. For example, it would not be appropriate to maintain human remains for mock casework ground truth reference material. Where ground truth solutions are not possible or practicable, witnessing by a second qualified analyst will be required to mitigate risk. This may be for all instances (such as with human remains), or with a defined subset.

Annual Case Review

Annual case review is a review of a sample of case files, including both forensic casework and database batches, in fulfillment of FBI QAS 3.4. The scope of this review will be determined annually and approved by the Technical Manager, in advance of the review. At a minimum it must include a representative sample of cases. For forensic casework, the case review must include these elements, which are not addressed in AR 7.7.6 performance monitoring:

- Following triage protocol for types of evidence sampled
- Following triage protocol for how items are sampled
- Following triage protocol for amplification decisions
- Correct calculation and reporting of STR single source statistics
- Correct calculation and reporting of STR mixture statistics
- Correct calculation and reporting of STR parentage statistics
- Correct calculation and reporting of Y-STR single source statistics
- Correct documentation and archiving of reports, notes, and raw data
- CODIS profiles are appropriately entered and documented
- Report language is complete and correct
- Amended reports are documented correctly (if applicable)
- All CODIS verifications are complete and correct

- CODIS communications are complete and correct

For database analysis, the case file review must include these elements, which are not addressed in OSAC performance monitoring:

- Batch documentation is complete and correct
- All batch documentation is correctly retained, including lab work documentation, batch upload reports, and electronic data
- “Good faith efforts” are followed appropriately

Professional Development and Continuing Education

Forensic FBI QAS Standard 16 / Database FBI QAS Standard 16

All DNA personnel (except for technicians) annually receive a minimum of 8 cumulative hours of continuing education specific to DNA, in accordance with the FBI QAS requirements.

Note: Literature reviews can count as one hour of continuing education per article toward the lab wide continuing education requirement but cannot be used toward the FBI QAS mandated 8 hours per year.

Continuing education is documented in the analysts training record in LIMS. The record is reviewed and approved by the discipline supervisor and the DNA technical manager. The following information is required in the record:

- Course title
- Documentation of attendance (may include certificates, agenda/syllabus, etc.). Shall include an attendance list for internal training
- Training date(s) and number of continuing education hours
- Evaluation of course (content, instruction, relevance, etc.)

Additionally, the following documentation is required for continuing education provided by lab personnel:

- A record of the presentation
- The curriculum vitae of the presenter

Programs based on multimedia or internet delivery require written documentation of approval by the DNA technical manager.

Documentation required specifically for internal and/or multimedia education is retained in the Forensic Biology discipline share or designated location.

Contingency Plans for DNA Technical Manager Vacancy and Fewer than Two Full-Time Qualified Analysts

Forensic FBI QAS Standard 4.1.6 / Database FBI QAS Standard 4.1.6

Contingency Plan for DNA Technical Manager vacancy

Pursuant to the FBI QAS, the laboratory must have a documented contingency plan if the technical manager position is vacated. The plan will be as follows:

- If a current staff member is qualified to serve as DNA Technical Manager, that individual will be appointed as an interim technical manager. If the laboratory has more than one qualified individual, the discipline supervisor will coordinate with top management to appoint an interim technical manager.
    - Time period: This individual will serve until a permanent replacement is hired.
    - Casework and database analysis: The laboratory may continue to do work and issue reports under this scenario.
- If no current staff members are qualified to serve as the DNA Technical Leader, the DNA Technical Leader from another laboratory will be hired to serve in an interim capacity.
    - Notifications: The NDIS Custodian and State CODIS Administrator must be notified within 5 days if no current staff members are qualified to serve as the DNA technical manager, and a contingency plan shall be submitted to the FBI's NDIS Custodian within 14 days of the vacancy, using the QAS Contingency Plan Notification Form.
    - Time period: The interim DNA Technical Manager will serve until a suitable replacement can be found.
    - Casework and database analysis: The laboratory may not begin new casework or database analysis until an interim technical manager is in place and FBI approval of the contingency plan has been received. Casework or database analyses in which DNA analytical procedures have been initiated prior to the technical leader's vacancy may be completed.

Contingency Plan for <2 Full-Time Employees who are Qualified Analysts

Pursuant to the FBI QAS, the laboratory must have a documented contingency plan if the number of qualified analysts falls below two full-time employees who are qualified analysts.

This policy affects both casework and database functions, and these functions must each be considered. If two full-time analysts are each qualified in both casework and database analysis, then this policy does not take effect.

- If either casework or database does not have two full-time qualified analysts, then those work functions are affected by this policy.
    - Top management will coordinate with the DNA Technical Manager to hire and train new analysts, re-assign previous qualified analysts working in other laboratory

disciplines, and/or notify customers of options for having analysis performed by a vendor laboratory without possible CODIS entry.

- Notifications: The NDIS Custodian and State CODIS Administrator must be notified if the number of database-qualified or casework-qualified analysts falls below two full-time employees, using the QAS Contingency Plan Notification Form.
- Casework and database analysis: The laboratory may not begin new casework or database analysis until FBI approval of the contingency plan has been received. Casework or database analyses in which DNA analytical procedures have been initiated may not be able to be completed if the number of qualified analysts falls below two full-time employees who are qualified analysts.

Outsourcing and On-Site Visits

Forensic FBI QAS standard 17 / Database FBI QAS Standard 17

Outsourcing of DNA casework must follow applicable requirements of FBI QAS Standard 17 (current version). Because details may vary between outsource contracts, this section of the Guidance Document will be updated as needed to reflect the procedures specific to the outsource contract.

This procedure has been updated to reflect the procedures specific to outsourcing with DNA Labs International.

Requirements to be met before vendor laboratory begins analysis.

In order for the Alaska Scientific Crime Detection Laboratory (AK SCDL) to maintain compliance with FBI QAS Standard 17 (current version), several requirements must be met prior to the vendor laboratory beginning its analysis of items which ultimately may be entered into CODIS. This manual defines procedures and the required documentation associated with Standard 17 compliance, for cases analyzed and reported at a vendor laboratory for the purpose of subsequently taking ownership of the vendor laboratory's work product, including but not limited to:

- Extracts which could later be amplified at AK SCDL
- DNA profiles which may later be entered into CODIS
- DNA profiles which may later be interpreted for compared against DNA profiles generated at AK SCDL.

Note: These procedures are not required for vendor laboratory analysis where the AK SCDL does not subsequently take ownership of extracts or DNA profiles.

Vendor laboratory compliance with FBI QAS and Accreditation Requirements (FBI QAS Standard 17.1)

- The vendor laboratory must provide documentation of compliance with FBI QAS standards and accreditation requirements of federal law.
- The AK SCDL Technical Leader will review the vendor laboratory's compliance with these standards as part of the on-site visit procedure (see below)
- The AK SCDL will retain documentation of audit and assessment records and certificates.
- The vendor laboratory will provide updated assessment and audit reports as they become available, for the duration of the contract.

The AK SCDL DNA Technical Leader will review and approve the technical specifications of the outsourcing agreement with the vendor laboratory before analysis begins. (FBI QAS Standard 17.2.1)

- The AK SCDL will retain documentation of the approved technical specifications.

On-site Visit Procedure for vendor laboratory performing forensic casework (FBI QAS Standard 17.4)

- The on-site visit must happen prior to the vendor lab beginning casework, and annually thereafter for the life of the contract.
- If available, the laboratory shall review and evaluate the most recent (within one year) on-site visit performed by a designated FBI employee.
    - Review of the on-site visit must include review of the vendor laboratory's compliance with federal and FBI QAS standards
- Documentation for the on-site visit must include:
    - The date the on-site visit was performed

    - A summary of the visit
    - The personnel who performed the on-site visit
    - Acceptance by the Technical Leader
- If no FBI on-site visit is available for review for a future contract, an on-site visit conducted by another NDIS laboratory using the same technology, platform, and typing amplification test kit may be sought for review.
- If no alternative is available, the Technical Leader will design and document an on-site visit protocol prior to performing an on-site visit.

The vendor laboratory must designate a point of contact

- The DNA Technical Leader or designee will be the point of contact for the AK SCDL.

The vendor laboratory must provide a sample case file for review

- This may be used to aid in developing a specific review procedure.

Technical review procedure for outsourced casework

Outsourcing of DNA casework must follow applicable requirements of FBI QAS Standard 17.3 Because details may vary between outsource contracts, this section will be updated as needed to reflect the procedures specific to the outsource contract.

The AK SCDL takes ownership of outsourced casework only when it will enter and search a DNA profile in CODIS from data generated by a vendor laboratory, or when it will use samples, extracts, or materials from the vendor laboratory for the purposes of forensic testing. Technical and administrative review of DNA casework will be conducted by qualified DNA analysts at the AK SCDL following the checklists provided either in this document or as separate controlled document.

If the technical reviewer finds any issues with an outsourced case, those issues should be brought to the attention of the DNA Technical Manager or designee, who will coordinate efforts with the vendor laboratory to resolve the issues. Profiles may not be entered into CODIS until technical issues have been resolved.

Together with the vendor laboratory review checklists, this section serves as guidance for the review of vendor laboratory data, reports, and documentation specific to cases submitted for analysis to DNA Laboratories International (DLI), for evidence submitted by law enforcement agencies.

TL = Technical Leader
FB sup = Forensic Biology supervisor
AK SCDL TR = qualified technical reviewer at Alaska Scientific Crime Detection Laboratory
The AK SCDL technical reviewer must be:

- Qualified in the technology (STR)
- Qualified in the platform (3500 xl genetic analyzer)
- Qualified in the typing test kit (GlobalFiler, including mixture interpretation)
- Participating in the AK SCDL's proficiency testing program

Initial Case Assessment

As a part of the initial assessment of each returned case, a FB sup or TL will check the following:

- All DLI documentation for cases, controls, and reviews is downloaded to the laboratory network.
- DLI issued a report for each submitted case, and the report has been added to the LIMS
- DLI releases results directly to the submitting agency as well as AK SCDL.
- Appropriate submitted evidence was analyzed for each submitted case
- If the case has potentially CODIS eligible data,
    - Relevant elimination samples must be requested from the submitting agency. This may be communicated verbally or via e-mail, but documentation must be maintained in the LIMS case file.
    - The RLS and any available case information must be reviewed to assess whether the profile would be suitable for CODIS entry. Additional information may be needed from law enforcement; this would then be documented in the LIMS case activities.
- Typically, the AK SCDL does not take ownership of outsourced casework that does not yield potentially CODIS eligible profiles. Cases identified as not having potentially CODIS eligible results will not routinely undergo technical review. Exceptions would be for cases with extracts that may require amplification at AK SCDL, or cases with DNA profiles to be compared against reference samples run at AK SCDL, regardless of CODIS eligibility.

Technical review by AK SCDL Technical Reviewer

Technical review of reports with possible CODIS profiles and their corresponding controls will be performed by AK SCDL TR, who reviews report elements as listed on Outsource Casework Review Checklist, including:

a. General: Batch/Central Log/ Control documentation includes a list of all applicable related cases. Any troubleshooting or quality issues raised in the batch controls are also appropriately documented.
b. Extraction: Each set of concurrently extracted samples includes at least one reagent blank. All reagent blanks are quantified. At least one reagent blank per extraction set is amplified. Any contamination issues with reagent blanks have been documented and satisfactorily investigated.
c. Quantitation: standard curves have appropriate R^2 and slope values (and/or other QA parameters, as applicable); NTC values are appropriate, and reagent blanks were quantitated.
d. CE: reagent blanks, allelic ladders, positive controls, and negative controls are amplified with expected results obtained; ILS is confirmed for all controls.
e. Case files: all tested items (or probative fractions) are addressed, the AK SCDL TR agrees with the reported conclusions, the conclusions are supported by the associated data, and the agency case and item numbers are correct.
f. If AK SCDL does not agree with or finds issues to be addressed in the report, AK SCDL TR notifies TL and works with DLI to resolve the concern.

CODIS entry of eligible profiles

1. Upload of profiles is performed by the AK SCDL TR, who checks the following items prior to CODIS upload:
    a. Case was not determined to be No Crime Occurred or Unfounded
    b. Evidence suitable for CODIS entry based on location, evidence type, etc.
    c. Correct specimen category identified.
    d. Profile is suitable for comparisons under current interpretation guidelines in Forensic Biology Procedure Manual.
    e. Profile meets MME/MRE eligibility requirements specified in the CODIS manual
2. AK SCDL TR uploads profile to CODIS (if appropriate).
3. AK SCDL TR completes their section of the case checklist. The checklist and the CODIS specimen detail report are submitted to another analyst, FB sup or TL for final case assessment.

Final case assessment

The final case assessment is typically performed by the TM or FB sup but can be performed by an AK SCDL CODIS qualified analyst, if designated. The final case assessment includes the following elements:

a. Review of CODIS entry, including the profile, specimen category, and NDIS/SDIS eligibility.
b. Scan the completed and reviewed CODIS specimen detail report to the case file in LIMS.
c. Notify the law enforcement agency that the profile has been entered into CODIS. This does not require a report but must be documented in the LIMS case file.
d. Ensure that DLI reports and case notes are retained in LIMS
e. Create and assign any CODIS hit letter requests that arise from CODIS entries. Hit letters will be released as they are completed.
f. Scan the completed review documentation to the case file in LIMS

Actions arising from technical review and/or CODIS entry:

- Any issues discovered during initial case review at AK SCDL or during technical review or CODIS entry will be routed through the TL to the technical point of contact at DLI. The TL will review DLI's responses to ensure that quality concerns are adequately addressed. If corrected reports are issued, either the TL or designee will distribute to the submitting law enforcement agency. If corrected case documentation or control documentation is provided, the TL will ensure that it is added to AK SCDL documentation archives, either in the LIMS if case specific, or the lab network if more general, as appropriate.

Overview of DNA Labs International documentation

All documentation necessary to complete a technical / ownership review must be submitted to AK SCDL and will be retained following review. This documentation typically includes:

- Report
- Case File (PDF containing case-specific data, control data, and administrative documentation)
- Electronic data (zip file; only needs to be examined if the above documents do not allow for full technical review)

Based on a sample case file provided by DLI, the Case File contains the following:

- Allele table for casework samples
- Search results against DLI employees
- Population frequency statistics printouts
- Extraction worksheets
- Quantification plate layout
- Quantification standard curve and quality metrics summary page
- Quantification results table
- Amplification plate layout
- Deviation request form was included in file adjacent to relevant procedure)
- CE plate layout
- Positive amp control electropherograms, with ILS
- Negative amp control electropherograms, with ILS
- Allelic ladder electropherograms, with ILS
- Reagent blank electropherograms, with ILS
- Casework item electropherograms, with ILS
- Amplification worksheet
- Preliminary Analysis worksheets (documentation of evidence images, biological screening, and sampling)
- Reagent worksheet
- Case Submission Form (contains customer specifications)
- Shipping information / Chain of custody
- Technical and Administrative review documentation

Notes:

- The sample case file negative control electropherograms did not include primer peaks, but checking primer peaks is a requirement for AK casework. If AK case file printouts of negative controls and reagent blanks do not include primer peaks, they will need to be checked by importing data into GMID-X.
- The sample case file included a deviation request form which was adjacent to relevant procedure documentation.

- The sample case file provided did not include a positive extraction control, but one is required for Alaska casework. Extraction positive control profile:

Loci	Allele 1	Allele 2	Allele 3	Allele 4
D3S1358	15	17		
vWA	15	18		
D16S539	11	13		
CSF1PO	11	12		
TPOX	8	10		
Yindel				
Amelogenin	X			
D8S1179	13	15		
D21S11	28	31.2		
D18S51	15	16		
DYS391				
D2S441	10	11		
D19S433	13	14.2		
TH01	7	10		
FGA	22	24		
D22S1045	15			
D5S818	12	13		
D13S317	8	9		
D7S820	10			
SE33	21.2	25.2		
D10S1248	14	15		
D1S1656	11	15		
D12S391	18	20		
D2S1338	23	25		

Management and Use of DNA Elimination Databases

As a part of its quality assurance practices, the laboratory maintains a searchable database of elimination DNA profiles. These profiles are retained in the CODIS software and/or the GeneMapper ID-X (GMID-X) for use with the profile comparison tool.

Purpose of the elimination databases

Elimination database profiles are searched against forensic casework and database profiles to increase the chance that contamination introduced during DNA analysis will be detected and will not be inadvertently uploaded to NDIS, as well as to assess the performance of samples used as internal positive controls.

Generation of the elimination database

The elimination databases consist of the following elements: reference samples (such as buccal swabs), the raw data generated from analysis of those samples, and the DNA profiles resulting from genetic analysis.

The elimination database is comprised of two groups

Group A - CODIS and GMID-X (STR profiles)

- Laboratory personnel who have routine direct contact with evidence / evidence outer packaging
- Laboratory personnel who have routine direct contact with forensic biology laboratory workspaces

Group B - GMID-X only (STR and Y-STR profiles)

- Laboratory personnel who have occasional contact with evidence / evidence outer packaging and/or forensic biology laboratory workspaces
- Law enforcement and/or medical personnel who have contact with or collect evidence
- Visitors and vendor staff who may enter the forensic biology laboratory workspaces
- Profiles attributable to consumable manufacturing staff
- Unattributable contamination profiles
- Positive control sample profiles (such as body fluid standards and positive controls associated with amplification kits)
- Volunteer samples for validation and QA/QC purposes

Individuals newly added to the elimination database are typed with the current STR and Y-STR kits, as applicable.

When amplification kits with additional loci are brought online, laboratory personnel who have routine direct contact with evidence / evidence outer packaging or who have routine direct contact with forensic biology laboratory workspaces should be typed with the new amplification chemistry and have their elimination profiles updated in the relevant elimination databases, as applicable.

Other previously typed individuals do not need to be re-typed with new typing kits except for specific instances of troubleshooting.

Management of elimination databases

Identification information forms:

- Prior to the upload of a profile to the elimination database, all individuals providing samples for the elimination database must receive and sign the Authorization to Obtain, Use, and Store Confidential Identification Information Form.
- Completed forms are submitted to a CODIS administrator or the DNA Technical Manager, who assigns the individual a unique code which is then noted on the authorization form.
    - Coding convention is that the sample will have a category and a number (such as STAFF101 or VENDOR34-NBS).
- Completed forms are retained on the secure laboratory network or designated secure electronic location. Profiles are not named with personally identifiable information, but they can be tracked back to the individual by way of the Identification Information Forms.

Personnel with read access to the elimination databases:

All CODIS-qualified authorized casework and database analysts have access to the staff profiles uploaded to the Combined DNA Index System (CODIS). All casework- or database-authorized analysts who have access to GMID-X software have access to the profiles in the Profile Comparison database.

Personnel with version control of the elimination databases:

Profiles are uploaded to the State DNA Index System (SDIS) by CODIS administrators and once uploaded, can only be modified by CODIS administrators. Profiles are uploaded to the GMID-X Profile Comparison database by DNA Technical Leader or supervisors and cannot be modified in the GMID-X software once they are uploaded.

Security:

Elimination samples are retained in secure forensic biology evidence storage space, with controlled access limited to authorized personnel. Raw data from analysis of these samples is stored on the secure laboratory network, which is backed up nightly and only accessible to authorized users. Both CODIS and GMID-X are password-controlled and require authorization prior to access.

Retention times:

Elimination database samples are stored indefinitely in the Forensic Biology evidence locker room, unless otherwise indicated on the consent form. Raw data from analysis of elimination samples is retained indefinitely on the laboratory network or other designated secure location. Because evidence may be analyzed well after initial collection, submission, or processing, elimination profiles are stored indefinitely in GMID-X. Removal of all material and information may occur if requested in writing by the individual. Profiles from Group A individuals are removed from CODIS when an employee is no longer employed by the laboratory.

Annual review of database contents:
As a part of the annual quality review, the GMID-X and CODIS databases will each be reviewed to ensure that all relevant individual profiles have been typed and uploaded to the appropriate databases, and staff profiles for individuals no longer working in the discipline are removed from CODIS.

Searching, evaluation, and resolution of elimination database matches:
Refer to the Forensic Biology Procedure Manual and CODIS Manual for procedures regarding search, match evaluation, and reporting matches to elimination database profiles.

Search policy:
For purposes of quality assurance, internal positive control samples and unidentified profiles are routinely searched against the elimination database in GMID-X. Profiles that cannot be confirmed against another related sample (such as a previously typed sample from the same individual or a body swab against an owner reference profile) are treated as unidentified profiles. This search happens prior to submitting a case for technical review and/or prior to NDIS upload, as applicable. Since these potential matches should be caught in GMID-X, the procedures are written specifically to the GMID-X

Match evaluation policy:
When data suitable for comparison is matched to an elimination database profile, or when an interpretable source of contamination cannot be identified, analysts will use appropriate quality assurance forms to document their evaluation of potential contamination. Depending on the nature and severity of the incident, this could be a Contamination Assessment Form and/or a Quality Assurance Review. Potential contamination issues should be resolved through retesting of evidence where possible.

Resolution of elimination database match policy:
When a match to an elimination database profile has been identified and attributed with certainty to a known individual, that individual should be notified, if possible. For individuals outside the laboratory (e.g., nurses, vendors, consumable manufacturers, etc.), this communication should be routed through discipline management. Within the laboratory, the DNA Technical Manager will ensure that appropriate personnel are notified. When matches to an elimination database profile are inconclusive, or when the exact source of point contamination cannot be isolated to a single vendor, no match will be reported.

Reporting of elimination database matches:
Reporting procedures in the FBPM are written for instances in which a source was identified before the report was released. For matches identified after the initial report has been distributed, the association must be communicated to the submitting agency.

Amplification Cycling Parameters

GlobalFiler
Program: **gf-cswk**
Max ramping mode is used for amplification

95°C for 1 minutes, then:

ramp 100% to 94°C for 10 seconds
ramp 100% to 59°C for 90 seconds
for 29 cycles, then:

60°C for 10 minutes
4°C hold

PowerPlex Y23 (~1:40 amplification time)
Program: **Y23-30cyc**
Max ramping mode is used for amplification

96°C for 2 minutes, then:

ramp 100% to 94°C for 10 seconds
ramp 100% to 61°C for 1 minute
ramp 100% to 72°C for 30 seconds
for 30 cycles, then:

60°C for 20 minutes
4°C hold

PowerPlex Y23 Direct Amp
Program: **Y-direct_26CYC**
Max ramping mode is used for amplification

96 C for 2 minutes, then:

94 C for 10 seconds

61 C for 1 minute

72 C for 30 seconds

For 26 cycles, then

60 C for 20 minutes

4 C soak

GlobalFiler Express
HOLD
95°C for 1 minute, then:

26 CYCLES of
94°C for 3 seconds
60°C for 30 seconds

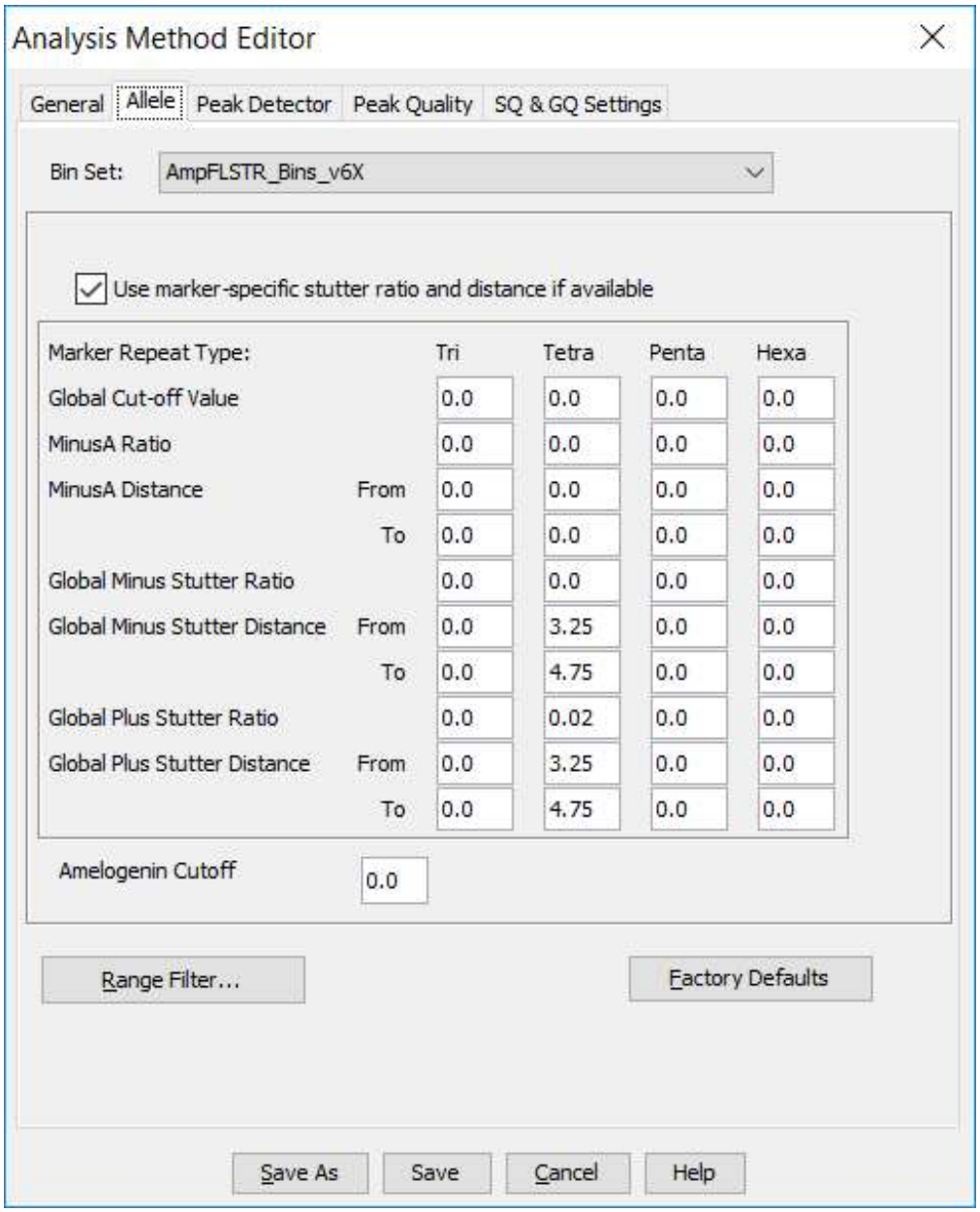
HOLD
60°C for 8 minutes

HOLD
4°C

Analysis Method Settings

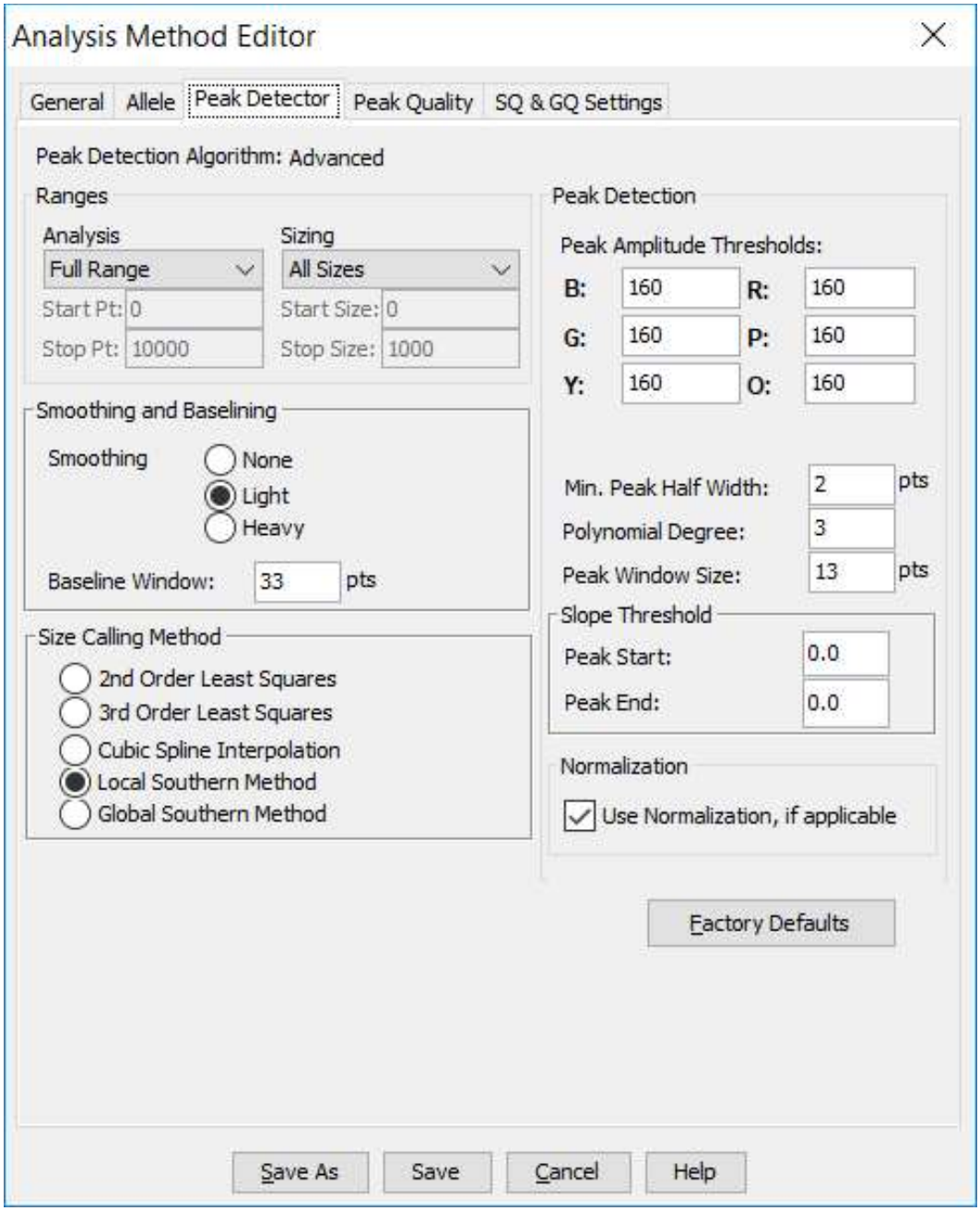
The settings for the Analysis Methods are viewed by selecting GeneMapper ID-X Manager under the Tools drop-down menu, then clicking on the Analysis Methods tab then double clicking to select a particular Analysis Method. These methods shall not be modified. New methods shall only be created or modified with permission from the DNA Technical Manager.

GlobalFiler Casework Allele Tab Settings



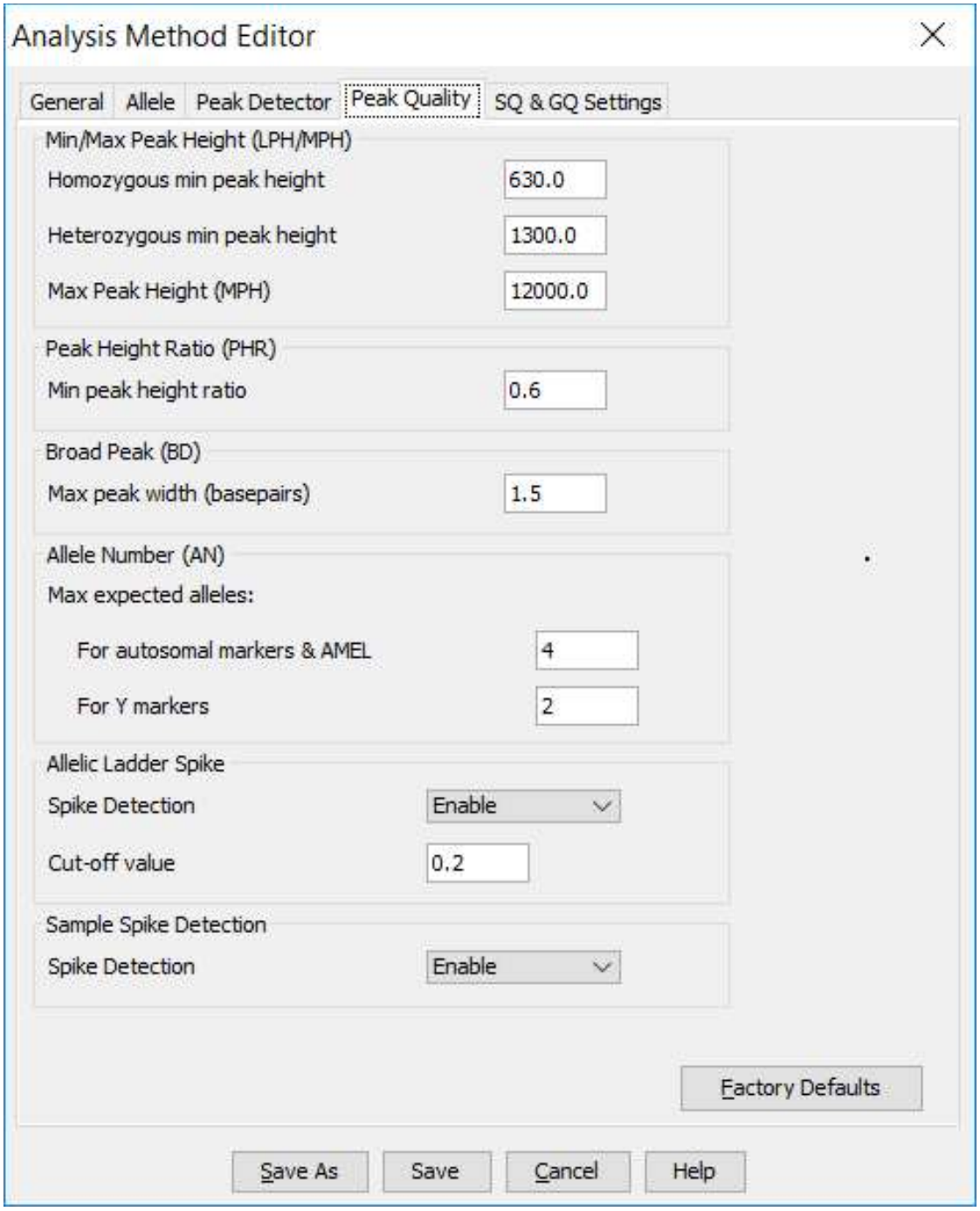
GlobalFiler Casework Peak Detector Tab Settings

The Analytical Threshold for all GlobalFiler casework analysis is 160RFU.



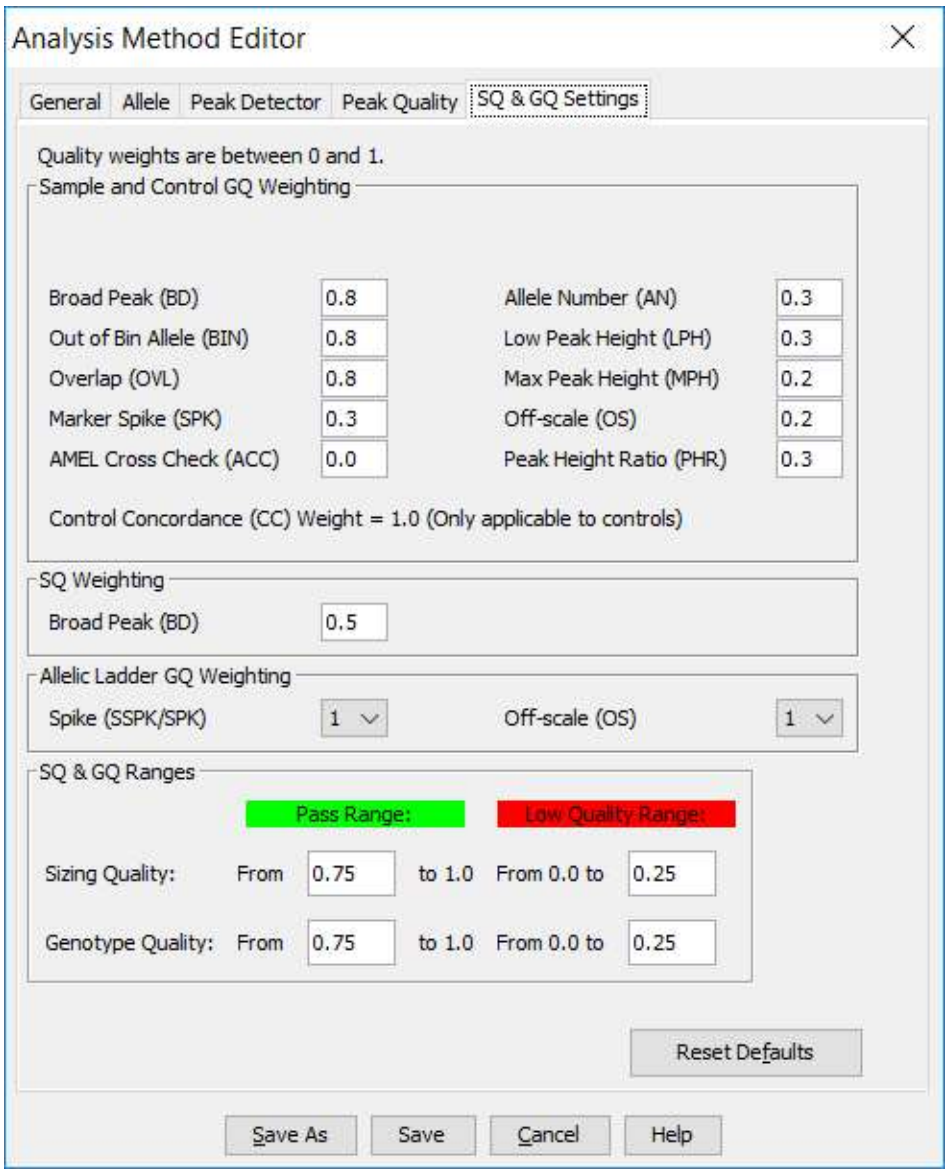
GlobalFiler Casework Peak Quality Tab Settings

These settings are not relevant in analysis of any samples where the sample type is set to Negative Control.

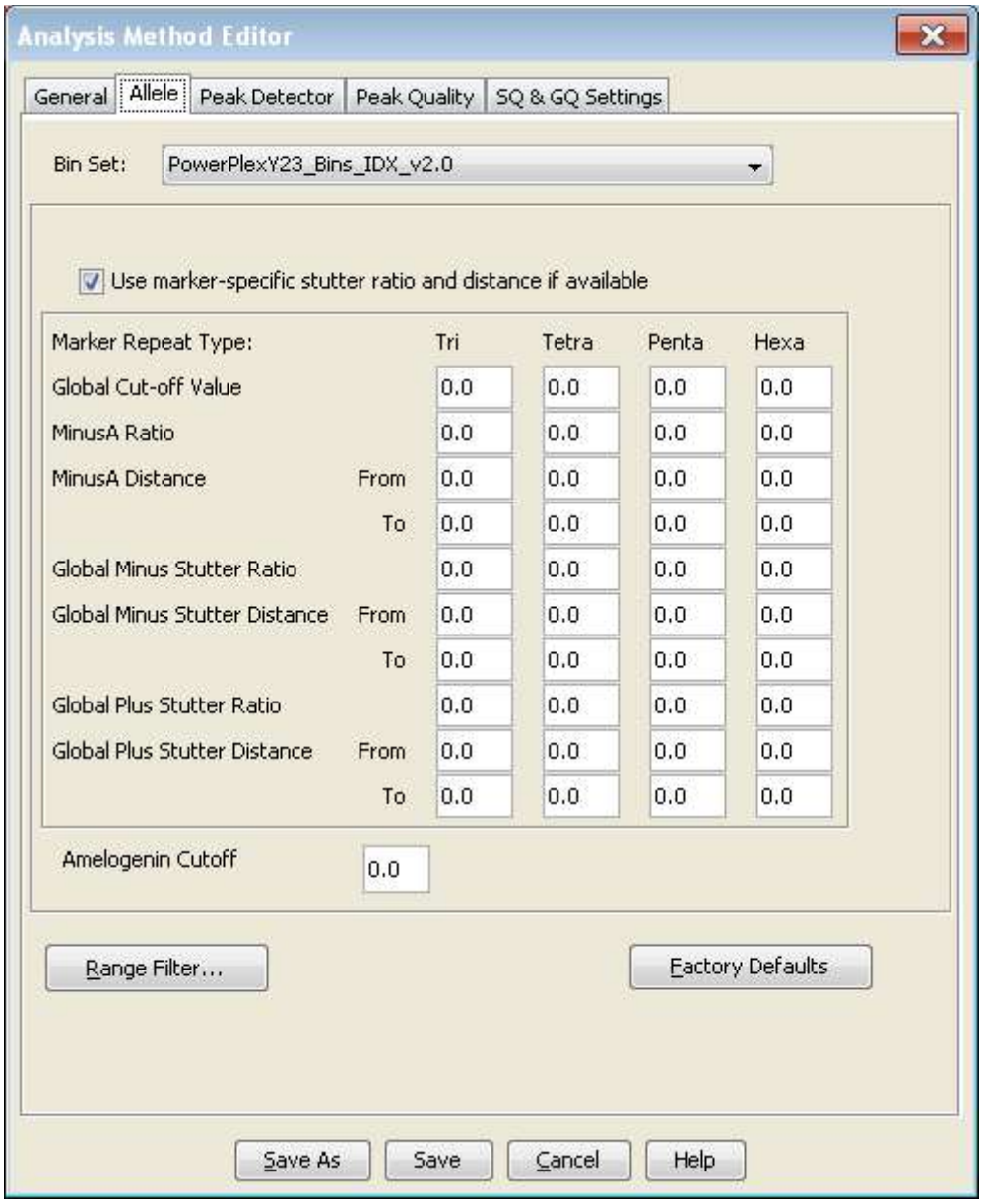


GlobalFiler Casework SQ and GQ Tab Settings

These settings are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

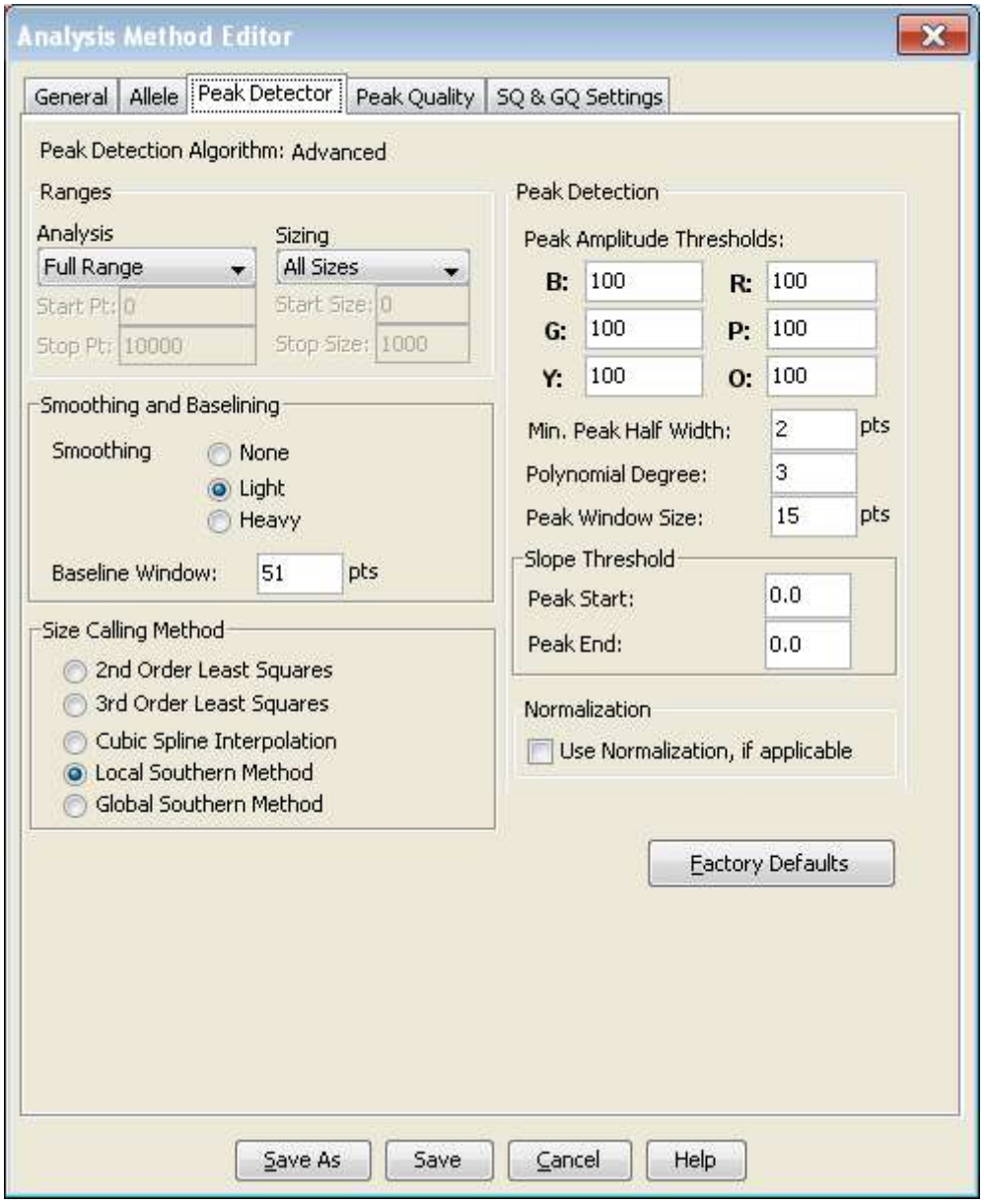


PowerPlex Y23 Casework Allele Tab Settings



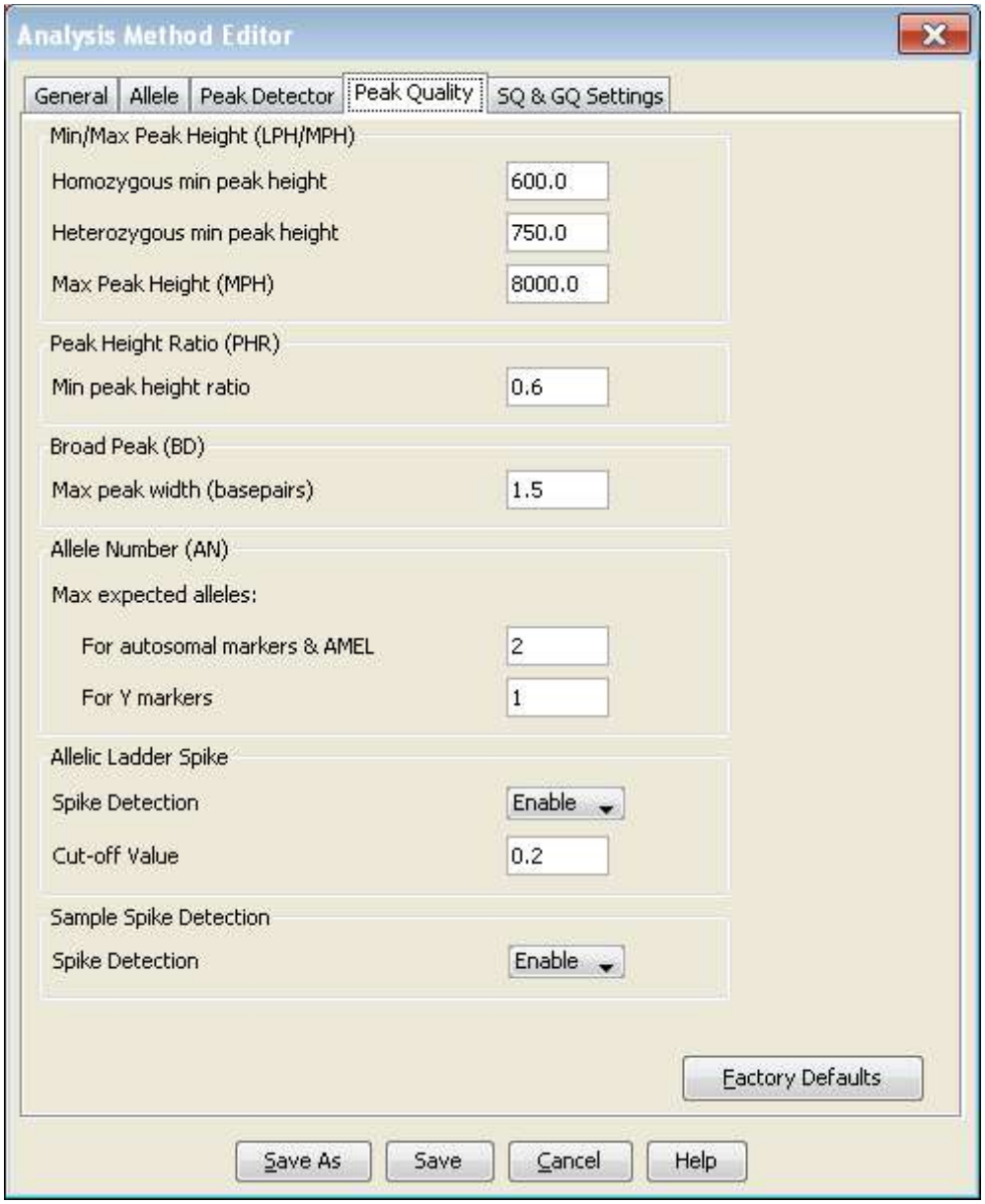
PowerPlex Y23 Casework Peak Detector Tab Settings

The Analytical Threshold for all Y-STR casework analysis is 100RFU.



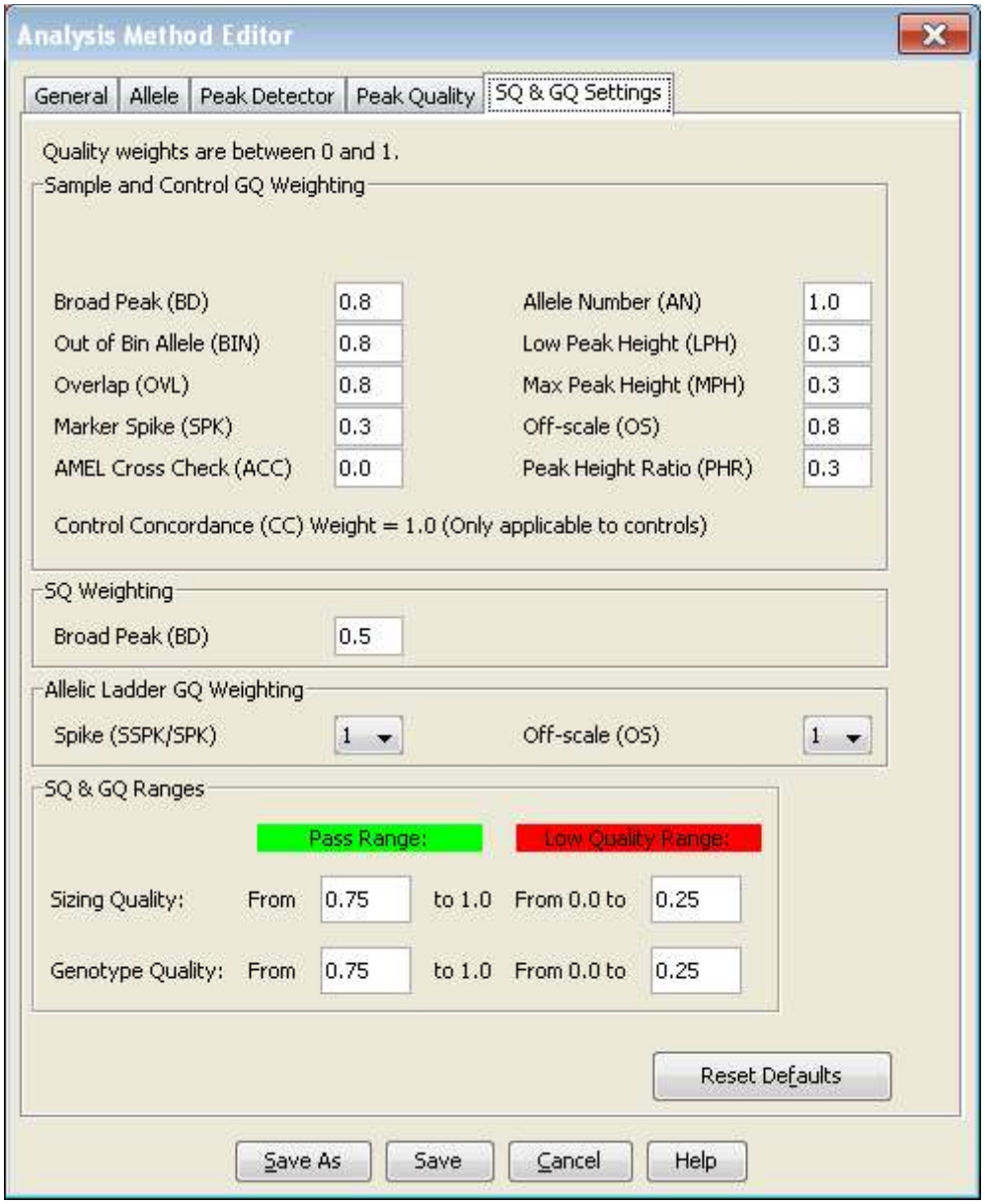
PowerPlex Y23 Casework Peak Quality Tab Settings

These settings are not relevant in analysis of any samples where the sample type is set to Negative Control.

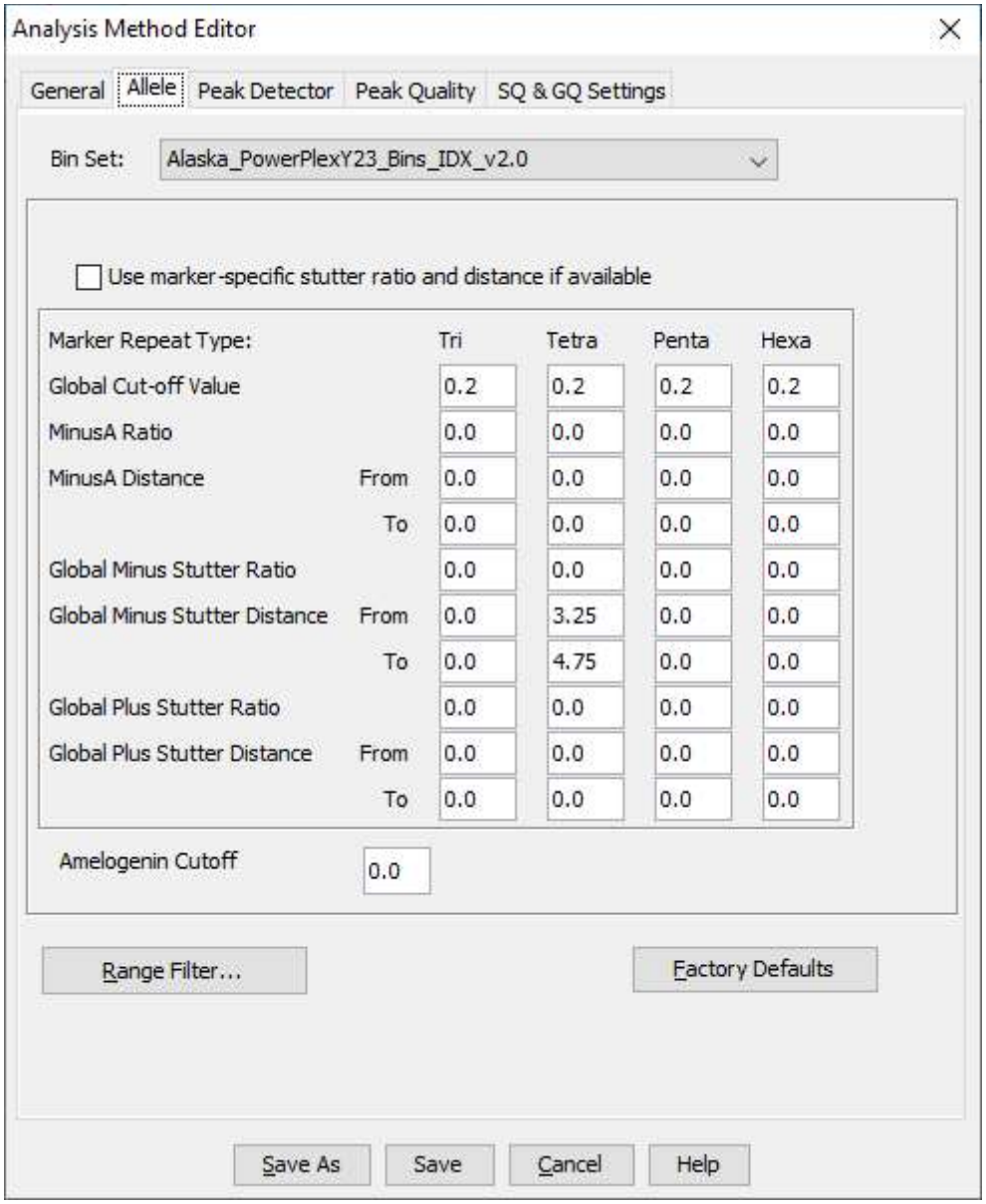


PowerPlex Y23 Casework *SQ and GQ Tab Settings*

These settings are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.

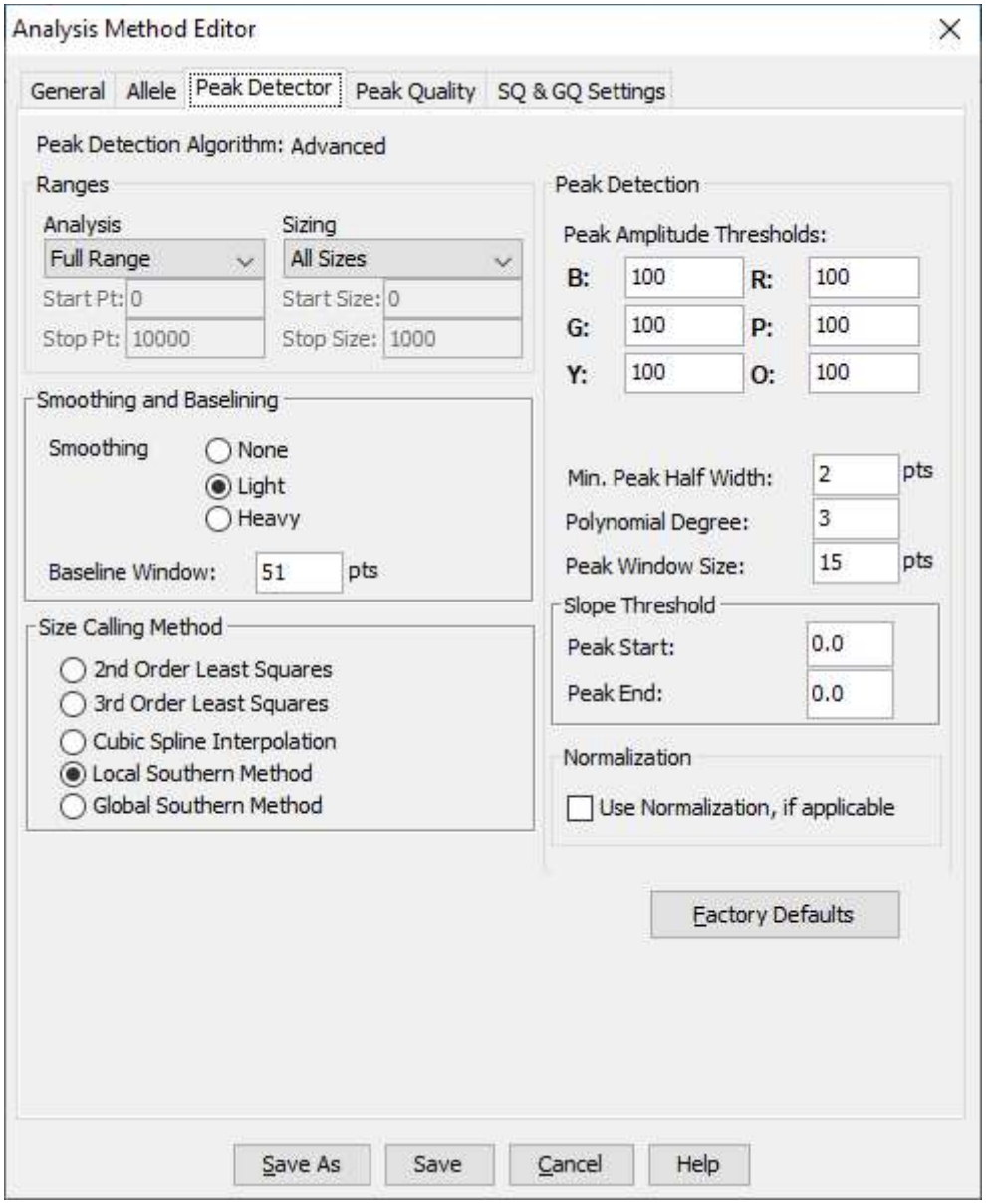


PowerPlex Y23 Direct Amp Allele Tab Settings



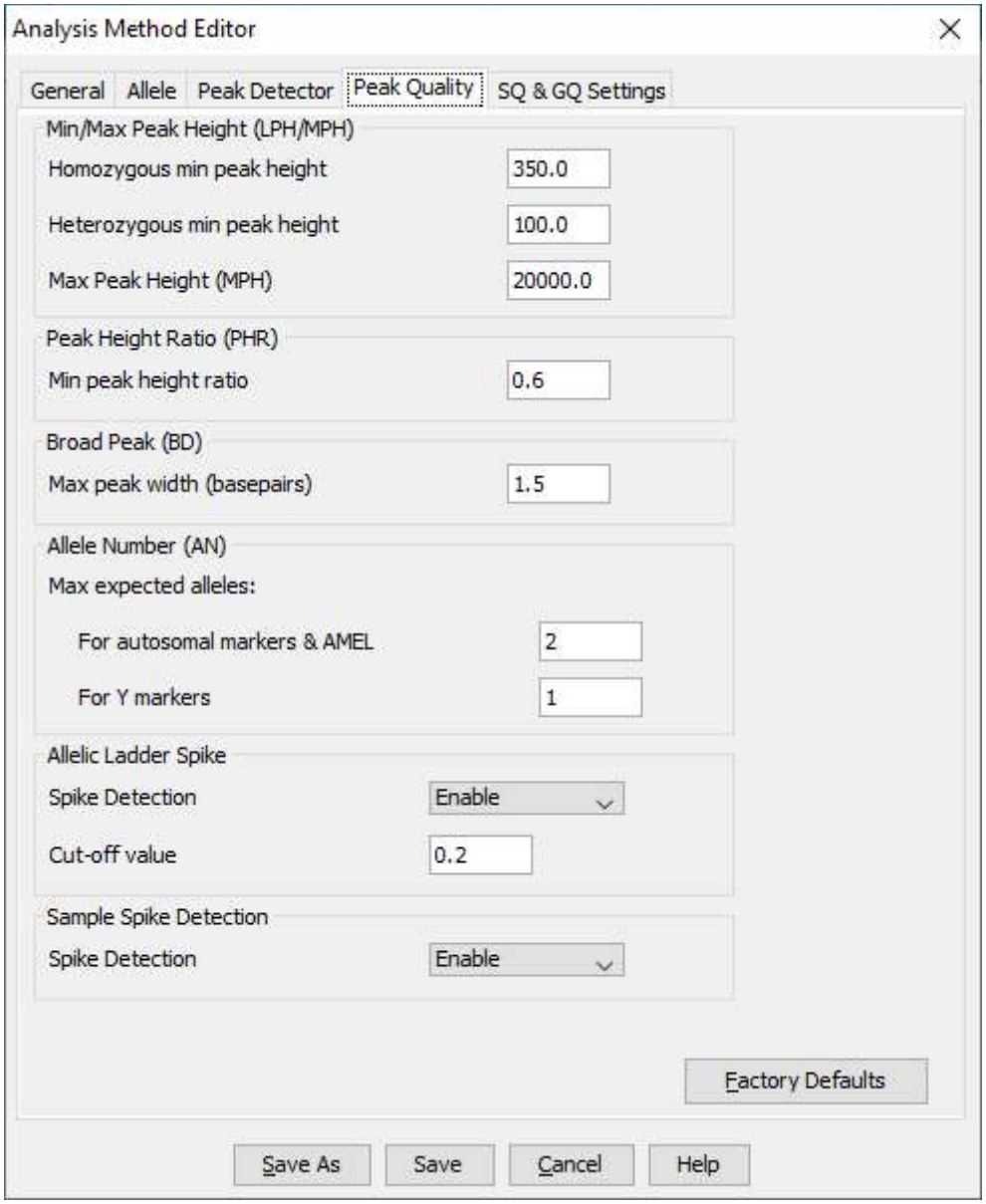
PowerPlex Y23 Direct Amp Peak Detector Tab Settings

The Analytical Threshold for all Y-STR analysis is 100RFU.



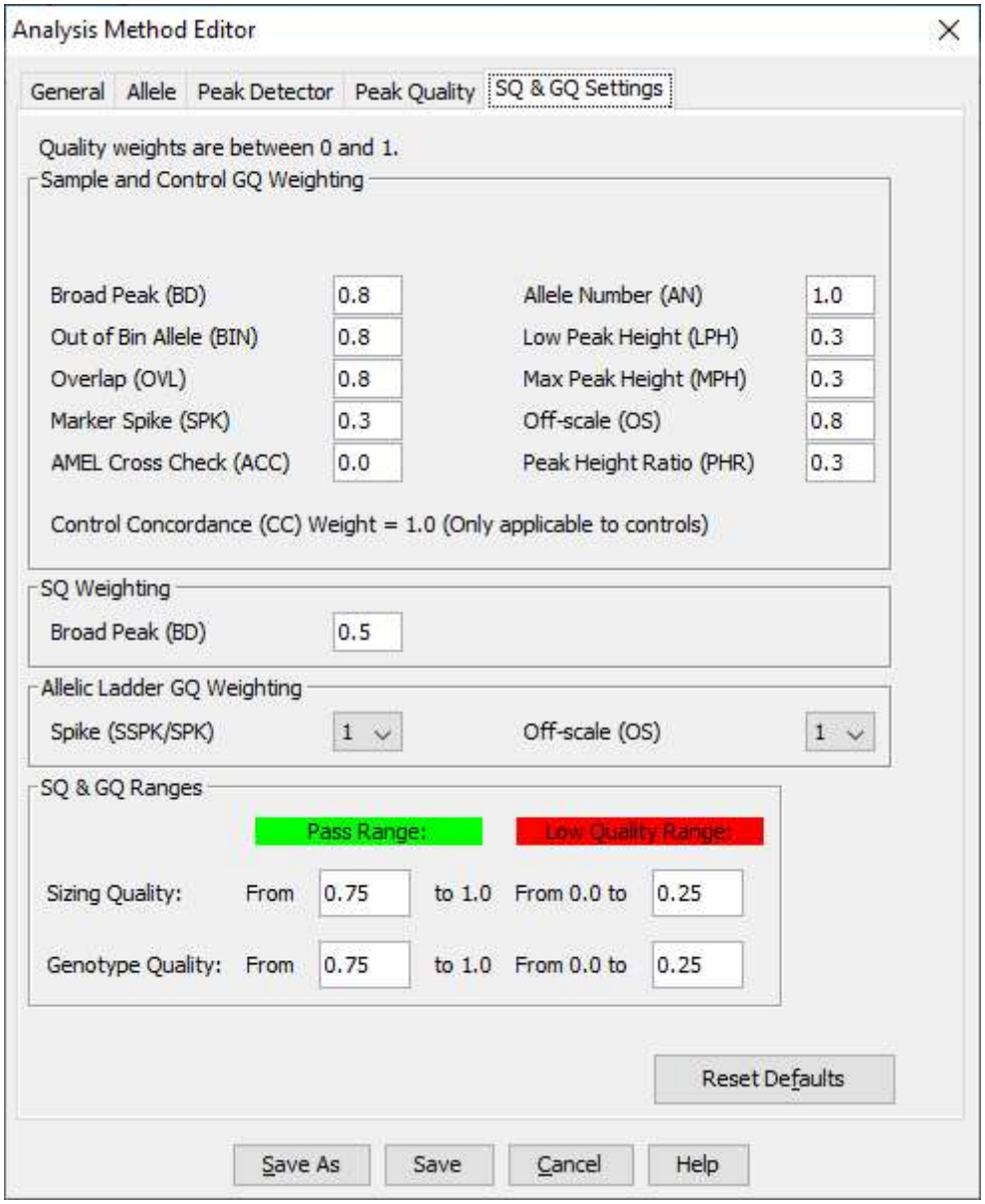
PowerPlex Y23 Direct Amp Peak Quality Tab Settings

These settings are not relevant in analysis of any samples where the sample type is set to Negative Control.

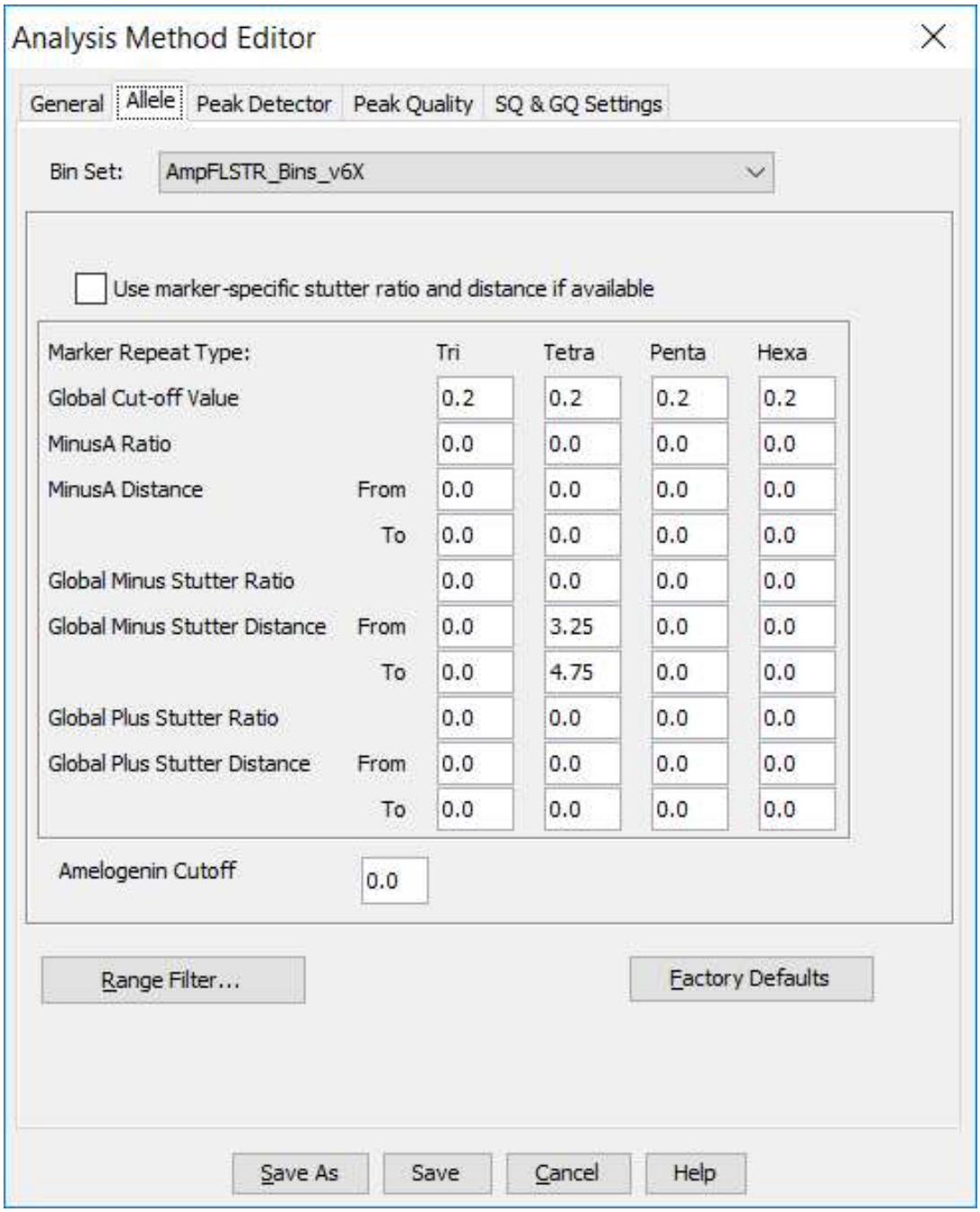


PowerPlex Y23 Direct Amp SQ and GQ Tab Settings

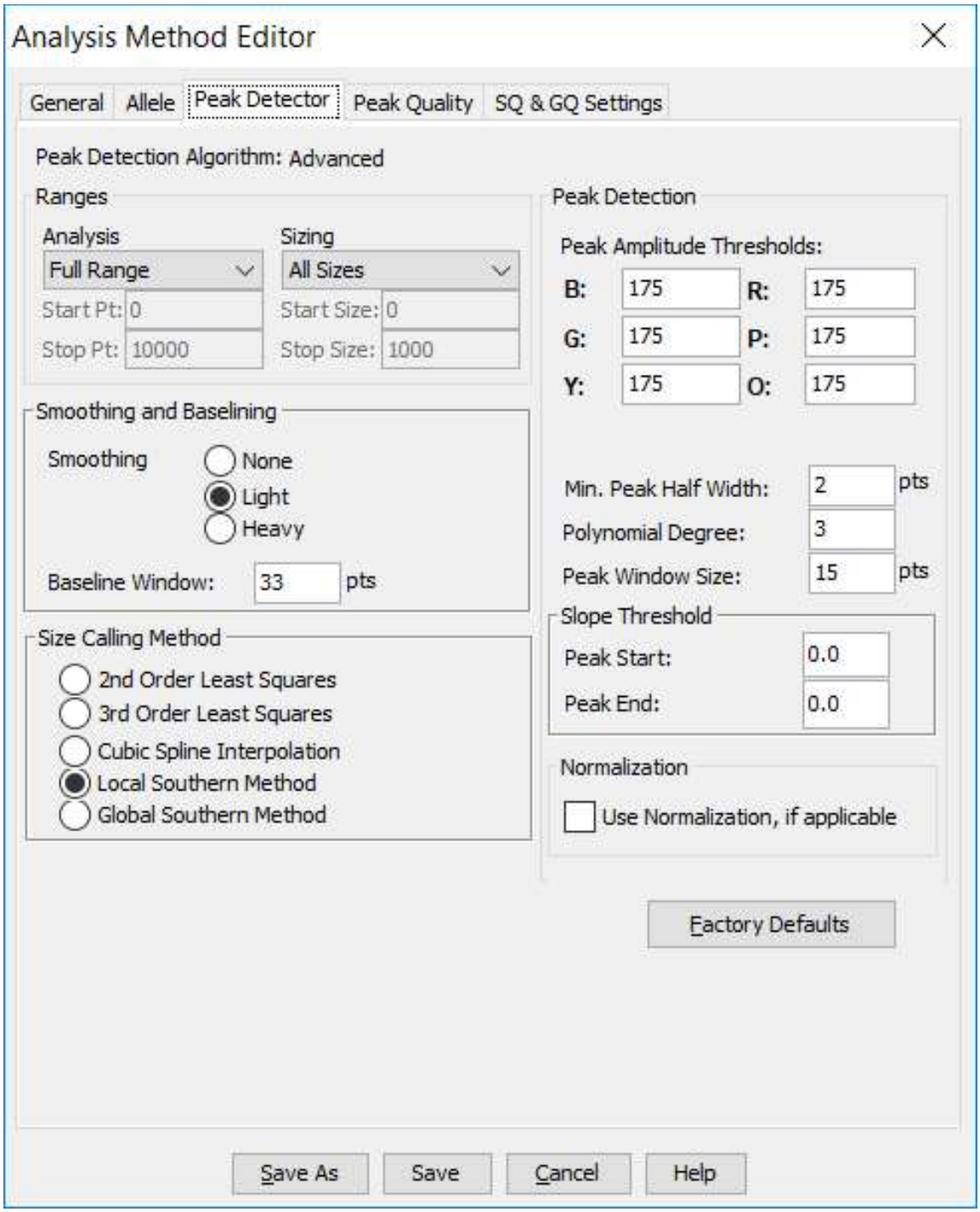
These settings are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.



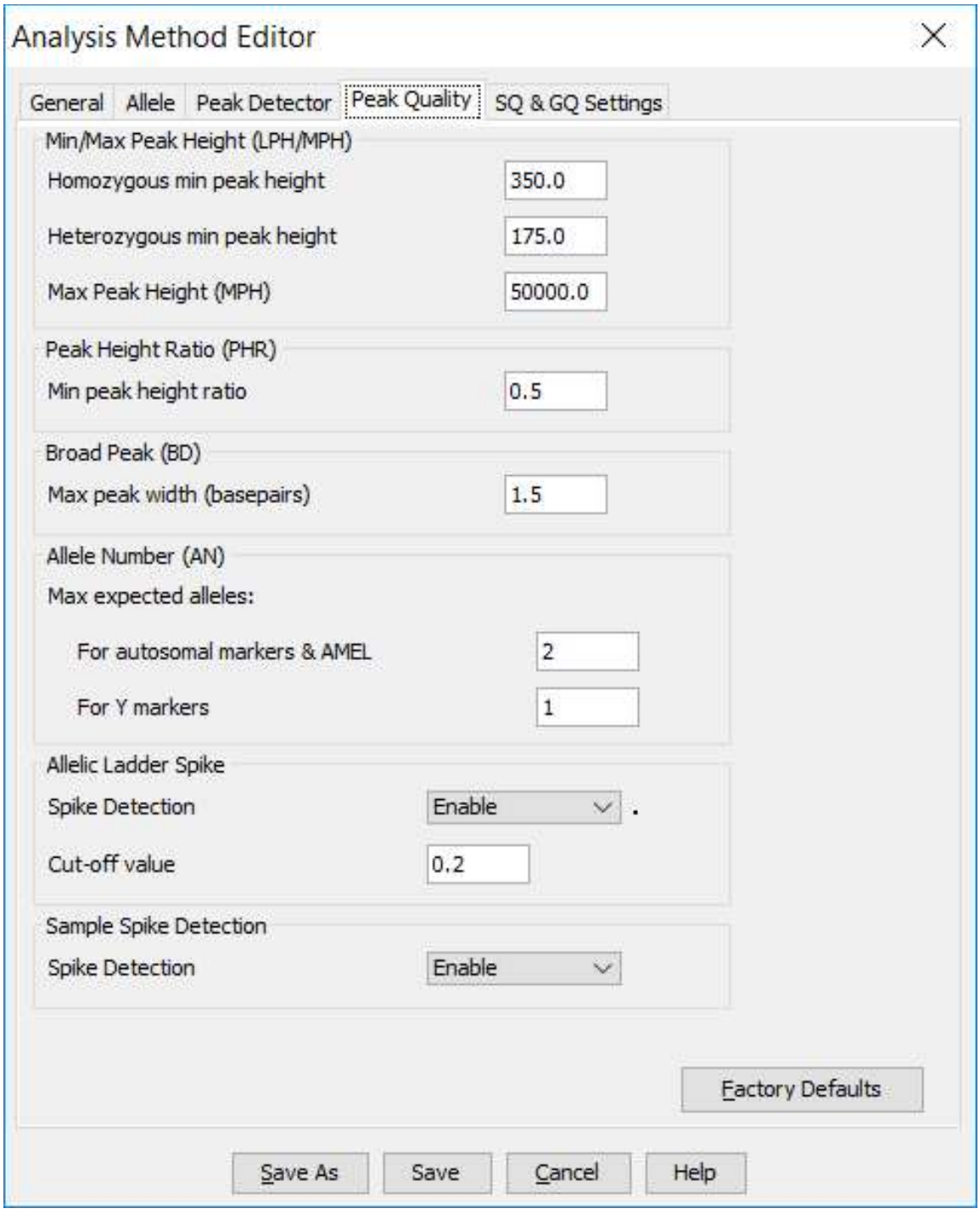
GlobalFiler Express Allele Tab Settings



GlobalFiler Express Peak Detector Tab Settings

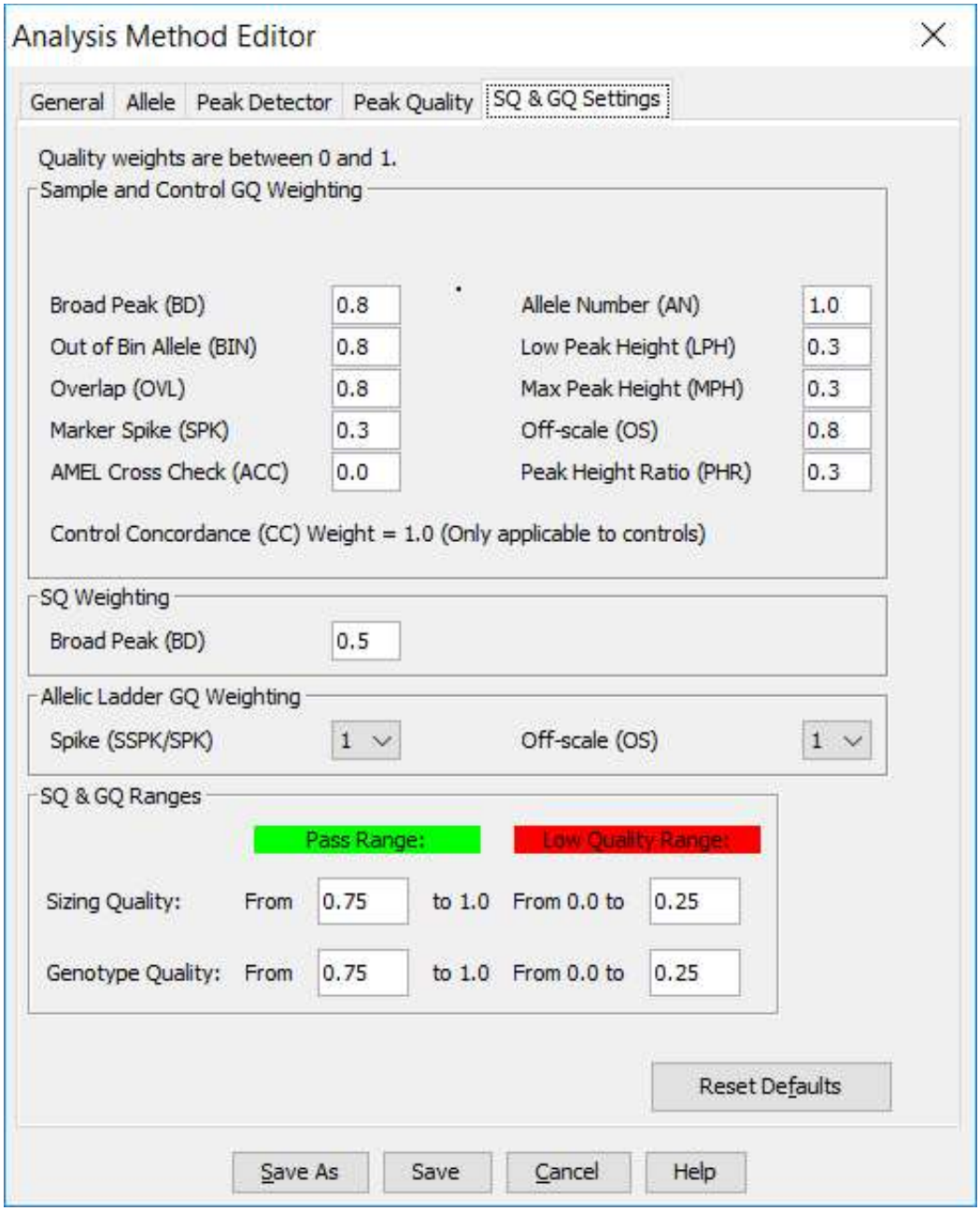


GlobalFiler Express Peak Quality Tab Settings



GlobalFiler Express SQ and GQ Tab Settings

These settings do not vary and are not relevant, as all samples are currently manually reviewed and interpreted, regardless of flagging.



RAPID HIT

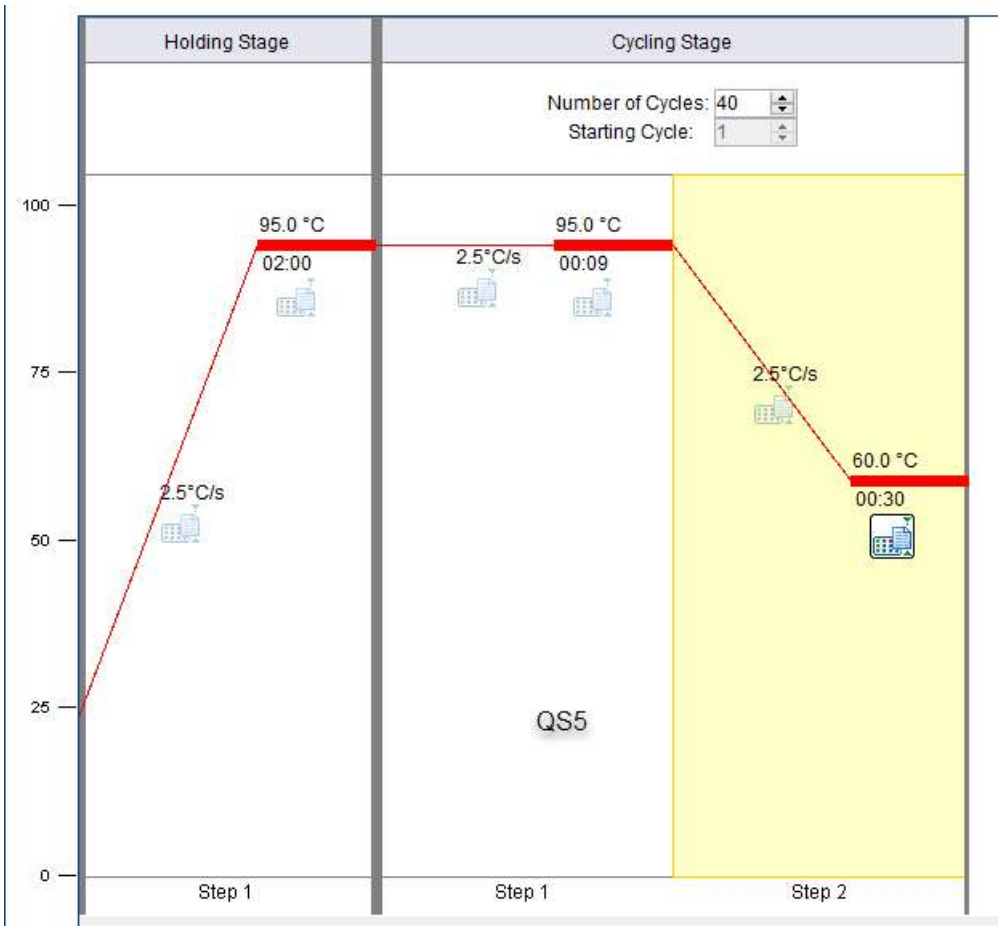
These threshold settings are programmed into the GeneMarker HID STR Human Identity Software used in conjunction with the RapidHIT instrument:

System Threshold	RapidHIT ID ACE GFE Sample Cartridge
Analytical threshold	35 RFU
Stochastic threshold (inconclusive homozygous or IHO flag)	All loci 91 RFU, except: TPOX = 105 Y indel = 35 DYS391 = 35 TH01 = 140 SE33 = 105 D12S391 = 105 D2S1338 = 105 D18S51 = 100 FGA = 100 D13S317 = 100
Minimum peak height ratio threshold (heterozygote imbalance or IMB flag)	40%
Stutter filters	20%
Locus-specific filter	20%
Ploidy threshold (maximum number of expected peaks)	2
Global filter (between loci)	20%
Minimum off-ladder intensity	30 RFU

Quantifiler Trio Cycling Parameters

(as defined in the Quantifiler HP and Trio DNA Quantification Kits User Guide)

QuantStudio 5 Instrument:



Reinterpretation of Data Typed with a Legacy Amplification Kit

Forensic FBI QAS standard 6.7 and 6.8

On occasion, the laboratory may be asked to revisit a case where analysis has been performed using a legacy amplification kit, defined as an amplification kit no longer covered by the current SOP (e.g., PowerPlex 16 or Profiler / CoFiler). Examples of this situation would include:

- Evaluation of a moderate stringency match in CODIS
- Submission of new questioned evidence in an old case
- Submission of new reference samples in an old case

Legacy data is suitable for comparisons if it can be used "as is" – in other words, as originally interpreted by the analyst. Typically, this would include:

- Single source questioned profiles
- Single source major component profiles from questioned mixtures
- Single source deduced profiles
- Two-source indistinguishable mixtures where a stochastic threshold was in place
- Reference profiles
    - While most reference profiles will not need to be retyped, it may be beneficial to retype reference samples when complex mixture interpretation is required in the current amplification kit.

Reinterpretation of legacy data is not permitted: Reinterpretation includes assessing or evaluating allele calls or genotype calls (including potential for drop out), changing assumptions used, or removing loci from statistical calculations.

- Owing to extensive changes in mixture interpretation policy over time, indistinguishable mixtures in legacy data with more than two sources as well as all indistinguishable mixtures interpreted without a stochastic threshold would require reinterpretation.
- Exceptions may be possible if the analyst and technical reviewer have been proficiency tested in the legacy kit within two years of the reinterpretation request. However, this would require documented and technical manager approved review of relevant validation studies and legacy SOPs.
- Consult with the Technical Manager first if a situation arises that involves legacy data requiring interpretation. If reinterpretation of legacy data is requested, the analyst should discuss options with the requesting agency, possibly including re-amplification of previously generated extract.
- Most CODIS profiles generated by legacy kits do not require reinterpretation and can be used "as is", as described above. However, some CODIS profiles from previously analyzed casework may require reinterpretation for comparison. When CODIS profiles that would require reinterpretation of legacy data are encountered, notify the CODIS Administrator or alternate CODIS Administrator. If the Administrator agrees that the profile would require reinterpretation for comparison, then the profile will be removed from CODIS.

Appendix A: Revision History

Location	Revision made
throughout	Corrections to grammar and spelling, minor (non-substantive) updates to language, addition of hyperlinks. Updated references to external standards documents.
Page 2-3	Added extracts to evidence definition. Added "during analysis" to the definition of work product.
Page 7	In heading starting "If quantification is performed…", changed 7500 instrument to quantification instrument. Updated "ROX" to "passive reference".
Page 9-10	Under Rejected electrophoresis data, updated to allow for documentation to be either in LIMS-DNA or on relevant electropherogram(s). Added bulleted section on Rejected observations.
Page 11	Updated location of master locker keys.
Page 12	Updated plan for literature review.
NA	Case management section relocated to Supervisor Working Instructions document.
Page 15	Annual quality review updated to include inventory of spaces not intended for long term storage of evidence.
Page 17	Removed reference to retaining LIMS-plus DNA refinement packet in SharePoint.
Page 35	Revised 1st bulleted statement under Group B and added 2nd bulleted statement
Page 36	Added hyperlink for volunteer consent form and updated sample nomenclature for coding of samples. Revised paragraph on retention
Page 52	Updated D18, FGA, and D13 locus-specific homozygote threshold values for Rapid HIT ACE GFE Sample Cartridges
Page 57	Removed 7500 cycling parameters image and added QuantStudio 5 cycling parameters image