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BATCH PAPERWORK & ANALYSIS

Analyst

1) When a new known is submitted to the laboratory for comparison to a case previously amplified with ID or IDP, the DNA profile is generated for the known.
2) The analyst will make comparisons of that known to all questioned samples in the case. Analyst will determine if the known is eliminated as the source of/contributor to the DNA profile, included as/CBE to the source of/contributor to the DNA profile, or if the comparison is inconclusive.
3) All questioned samples to which the known has not been eliminated as the source of/contributor to must be re-analyzed, using GeneMapper, using an analytical threshold of BGYR = 50rfu. Obtain the batch paperwork in which these samples were previously analyzed, if applicable (older cases with Identifiler might have been processed prior to batches being established).
    a. Add all appropriate questioned samples, all associated negative controls, and all associated ladders into GeneMapper project. (These files will most likely be in the “Completed” folder, in “3130 Nuclear DNA Casework & QC” folder, on the U-drive). Due to the size of the completed folder, one may also move data from the completed folder to analyst specific “to be analyzed” folder prior to importing into GeneMapper. If this is done, ensure that they are returned to the completed folder when analysis is finished. Archived data can be found on the DNA Server (U drive) or on the archive CDs. A new folder will need to be made for reanalysis and placed in the completed folder.
        i. The positive controls do not need to be brought in or reanalyzed, but you do need to look at the already created profiles to verify that they had previously worked as expected.
    b. Analyze data with appropriate analysis method containing the proper range, encompassing all size standards for all samples, and BGYR amplitude thresholds set to 50. (For IDP samples, this will be the same analysis method used in the past to create the input file for the ID+ Stutter Filter Macro.) Analyze data with either “Identifiler_Plus_Panels_v1” or “Identifiler_CODIS_v1.1”, depending on if the data was amplified with Identifiler Plus or Identifiler, respectively. All other settings will remain the same as the previous analysis.
    c. Save with the following file name format: Kit-AT-initialsdate-Allele. (AT is analytical threshold; date will be the date you’re doing analysis.) For example, IDP-50-JBE060617-Allele. (Allele is to distinguish that this file is for the allele table.) If you will be analyzing more than one project in a day, add -1, -2, etc to the end of this name.

d. Use the previous analysis as a guide for click-offs. Click-off all peaks/artifacts previously clicked off. Analyze data for possible artifacts between 50 & 74 RFU, which would not have previously been clicked off. Analyze data for stutter peaks between 50 and 75 rfu that were not previously called, but are at or below the stutter threshold for that allele. Make and record additional click-offs appropriately on original Injection Analysis Worksheet.

e. Analyze negative controls for peaks between 25 and 49 RFUs. If any peaks are noted, record on QR sheet. Follow SOP 31.5.3 when dealing with contaminated controls.

f. Print out electropherograms for negative controls and questioned samples. Compare questioned samples to previously reported out electropherogram. The only differences should be that asterisk peaks are now called peaks. All alleles should be identical.

g. Note that all controls/standards performed as expected on original injection sheet, by adding additional column of checks (next to space to right of previous checks), with initials and date. "N/A" the "Cross-Comparison to Batch" line.

h. In the "Genotypes" tab, export "GeneMapper Analysis Table" (see below to create table) to the analyst specific "Analysis Export File" folder on U-drive in the "To Be Analyzed" folder. File name will have same name as project name. This text file will be used for the Contributor Estimator and the Allele Table Macro.
    i. Check the following columns for the "GeneMapper Analysis Table": Sample File, Marker, Allele, Size, & Height. Set the number of alleles to 39. Uncheck the "Keep Allele, Size, Height, Area, Data point, Mutation and Comment together" box. This table will be used for the "Allele Table" on the report. This can replace the previous "Forensic Analysis Table", and will be the only table needed in table settings for GeneMapper. All tables exported from GeneMapper will be in this format.

i. Using the GeneMapper Manager, export the saved project to a folder where one of the previously analyzed data projects resides. If this can be exported to a variety of different folders, due to samples analyzed coming from more than one project, note on batch paperwork, next to your new analysis, in which folder this file resides. This will allow your TR to easily find the project to import it. Start a new project to close this project out.

j. Data will now need to be analyzed with stutter filters off. To do this, new panels and bins are necessary, and the creation of a new analysis method (only needs to be done once, then will be in your GeneMapper settings, if done, proceed to k):
    i. In Panel Manager, import panels, then bins for "Identifiler_Plus_Panels_No-ST-Filter" and "AmpFLSTR_Panels_No-ST-Filter".

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ii. In "GeneMapper Manager, under "Analysis Methods" tab, click on the method previously used to analyze IDP data and click "Save As", and re-name IDP-50-noST.

iii. Double-click the new method to open Analysis Method Editor. In "peak detector" tab, make sure all peak detection amplitude thresholds for BGYR are set to 50. In "Allele" tab, make sure bin set is the No-ST-Filter set just imported.

1. This will need to be done for IDP and ID if analyzing data obtained from both kits.

k. Bring in all files that were previously analyzed in step a. Re-analyze data, now with either IDP-50-noST analysis method / AmpFLSTR_Panels_No-ST-Filter panels for IDP, or ID-50-noST analysis method / AmpFLSTR_Panels_No-ST-Filter panels for ID. Using the analysis worksheet as a guide, click-off the artifact peaks (not stutter peaks) clicked off in the previous project. Remember to keep high stutter when applicable. Save file with the same name as before, but with –STRmix at end instead of -Allele, for example, IDP-50-JBE060617-STRmix. Although controls need to be brought into this project, click-offs for the controls do not need to be repeated.

l. Export the table, as done with "Allele" project. This will be the text file used for STRmix analysis.

m. Using the GeneMapper Manager, export the saved project to the same folder selected for the "Allele" project.

4) Use the "Project Comparison" tool to make sure click-offs in both projects are identical (with allowable exceptions noted in "comments" section).
5) Create a contributor # estimation (QR-302) sheet for each sample. Note: Generally the use of any non-artifact peaks below AT (25-49rfu) is added to this worksheet. With a single source sample, this worksheet is still used; comments are added as to the determination of number of contributors.
   a. Check the box in the "Notes" section if peaks below AT were used.
   b. The "minimum # of male contributors line" will be greyed out for IDP data.
6) Fill out new DNA QR-4A sheet(s), attach to batch (es) (if applicable) and new paperwork, and give these to Technical Reviewer for review.

Technical Reviewer

1) Import analyst's "Allele Table" project into GeneMapper then open that project. Guidance to where this project resides may be on the analysis worksheet.
2) Review questioned samples and negative controls. Note any differences. Check that:
   a. Correct analysis template is used.

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   b. Analyst's assessment of size standards, ladders, controls, and potential contamination are appropriate.
   c. All called peaks appear to be true alleles.
   d. All deletions/edits made are appropriate.
3) Review all new paperwork (sample electropherograms, control electropherograms, number of contributors sheets, project concordance sheet) and all additions to old paperwork (new edits, new controls/standard performance). Add a new set of checks in a column on the injection sheet for controls and standards, to the right the previous 2nd analysts checks, and initial and date.
4) Fill out, and initial and date DNA QR-4A.
5) Give batch to AR.

Administrative Reviewer

1) Review all batch paperwork.
2) Fill out, initial, and date DNA QR-4A.
3) Give batch back to analyst.

Analyst

1) Review and make any corrections.
   a. If additional click-offs need to be made, or click-offs need to be undeleted, do so in both STRmix and Allele Report project. Re-save and Re-Export both projects. Re-export both text files. Rerun project comparison macro. If appropriate, re-run affected contributor number estimates.
2) Initial and date DNA QR-4A. Return batch to Technical Reviewer.

Technical Reviewer

1) Review any changes made by analyst.
2) Add batch name to spreadsheet on U-drive.
3) Initial and date DNA QR-4A.
4) Return batch to Analyst.

Analyst

1) Make photocopy of injection sheets for case jacket in batch, if applicable. This, along with the original questioned sample electropherograms, and contributor number estimator, are for the case jacket.
2) Scan batch paperwork (not containing sample electropherograms & contributor number estimator sheets) and save PDF file to appropriate folder on u-drive.

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3) Give batch paperwork to TR to review scan.

Technical Reviewer

1) Ensure all pages scanned and PDF file on u-drive is complete and accurate.
2) Add initials to batch paperwork spreadsheet.

CASEWORK

Analyst

1) Deconvolute (see DNA SOP 22) all questioned samples just reanalyzed in STRmix with appropriate knowns conditioned*, and appropriate knowns being compared to deconvoluted profiles.
    a. The file will automatically save onto the server, under F:\results.
    b. Print out pages 1 and 3 of the advanced report.
    c. Scrutinize advanced report for any problems with the deconvolution.
    d. Fill out DNA QR-303 for the 2nd diagnostics for each deconvolution.
    e. Move the file in the F:\ results folder to your folder in F:\results.
    f. Create a folder in your folder for this case, named with the case number in format DSS-XX-XXXXXX, or ID-XX-XXXXXX, and move that folder into this newly created case specific folder.
2) Review case jacket, write report.
3) Give case jacket to TR.

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Technical Reviewer

1) Review case jacket, report, and make all appropriate corrections.
    a. If there is a disagreement with an elimination comparison, the analyst re-analyzes that sample and runs the profile through STRmix. Repeat “Analysis” portion of work instructions.
2) If there is a need to review more of the Advanced Report than is in the case jacket, it can be found on the STRmix server, F:\Results in the analyst’s folder, in the folder specific to this case.
3) Give case jacket to AR.

Administrative Reviewer

1) Review case jacket, report, and make all appropriate corrections.

   a. If there is a disagreement with an elimination comparison, the analyst will re-analyze that sample and run the profile through STRmix. Repeat "Analysis" portion of work instructions.
2) If there is a need to review more of the Advanced Report than is in the case jacket, you can find it on the STRmix server, F:\Results in the analyst's folder, in the folder specific to this case.
3) Give case jacket back to analyst.

Analyst

1) Make all appropriate corrections to report and case jacket.
2) If either the TR or AR disagrees with an elimination call, that sample must be re-analyzed with the new AT, and run through STRmix. Repeat "Analysis" portion of Work Instruction.
   a. If the conclusion is still an elimination, the "elimination by STRmix" report wording is used, and not "elimination by analyst"; wording will be slightly different. See report template.
   b. If the conclusion is not an elimination, report out results based on LR.
3) Print out copy of final report.
4) Move the STRmix results from analyst folder to the completed folder on the F:\ drive.
5) Give the case jacket to TR for correction check.

Technical Reviewer

1) Ensure all corrections are accurate, sign report.
2) Check to make sure STRmix data has been moved to F:\Results\Completed.
3) Give case jacket to AR for correction check.

Administrative Reviewer

1) Ensure all corrections are accurate.
2) Give case jacket back to analyst.

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*If knowns, previously analyzed, and already in the case jacket, need to be conditioned to this sample, the text file may need to be re-exported from GeneMapper so that it is in the proper format for STRmix. If so, please do the following:

1) In GeneMapper, open GeneMapper Manager.

2) Import analysts project by browsing to folder where GeneMapper project that was previously exported resides (most likely in the U:\ 3130 NUCLEAR DNA CASEWORK & QC \Completed folder).
3) In the Genotypes tab, export the "STRmix Table" to location where all knowns projects are exported (U:\3130 NUCLEAR DNA CASEWORK & QC\To Be Analyzed_Exported Known Projects). Add "-STRmix" to the end of this file, since there will most likely already be a file with this name already in the folder).
4) Close out of GeneMapper. There is no need for reanalysis, printing, or noting on previous worksheets.

Note: Knowns run with F6C can exported in GeneMarker, and be compared to IDP profiles in STRmix. However, this is for LRs only. Currently, there is no way to condition a F6C known to an IDP profile.

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