DNA WI-34 Analysis Workflow for Questioned Samples

Document ID: 5159

Revision: 10

Effective Date: 3/9/2022

Status: Retired Page 1 of 7

Approved by Director: Dr. Guy Vallaro

BATCH PAPERWORK & ANALYSIS

Analyst

- 1) Bring all data files into GeneMarker and analyze using GF CTDSS.
- 2) Save GeneMarker project to file folder that HID files are in (U:\3130 NUCLEAR DNA CASEWORK & QC\To Be Analyzed).
- 3) Analysts may utilize QR-342 as follows: export text file to your "Analysis Export File" in your "To Be Analyzed folder". Use exported text file to create "Supplemental GlobalFiler Stutter" Filter" worksheet, DNA QR-342, to use as a guide for stutter peaks with low template/n-2 stutter contributing. If the supplemental stutter filter is not utilized (e.g., if the profile is single source or if you feel that the filter is not needed), still print out the QR-342 worksheet and note that the batch had no N-2 or low template stutter concerns.
- 4) Analyze samples, adding comments for all click-offs. Click-offs should mostly be dye-artifacts, raised baseline, pull-up peaks, and stutter peaks that have n-2/low template stutter contributing. 'St' is a sufficient comment for all stutter types.
- 5) If a sample needs to be re-injected, or if for another reason you are not reporting out a sample, disable the sample in GeneMarker, noting the reason on QR-325 and in GeneMarker.
- 6) Re-save GeneMarker file when all click-offs have been made and all appropriate samples have been disabled.
- 7) Re-export report as a text file to your "Analysis Export File" in your "To Be Analyzed" folder, you can save over the file created in step #3. This text file will be used by the Concordance Checker, Cross-Comparison/Staff Search, Contributor Estimator, Deconvolution workbook, and Allele Table macros.
- 8) Run concordance checker (DNA QR-37) if positive controls are present, using text file created.
- 9) Print out e-grams from samples and controls. If analysis is done remotely, printing may be done at a later time.
- 10) Repeat steps 1 thru 9 if there are multiple injection sheets.
- 11) For each sample project, start a new project by bringing in the same data files into GeneMarker, and analyze them using the GF CTDSS STRmix template. Save GeneMarker project with identical name as before, but with "STRmix" added to the end.
 - o **OR** you can re-analyze your data from the current project by pressing the green play button again. A message will come up asking if you want to re-call the size standard. You want to check the box for "Call Size Again" and click on "Apply to all". Don't forget when you save this file to save it with STRmix added to the end.
- 12) Disable all samples that you disabled in the previous project.
- 13) Using your analysis on the e-gram peak tables as a guide, click off all the same peaks you just clicked off with the following exceptions (these exceptions will come up as non-concordances when you run your project comparison, to be noted in the comments section, with the exception of stutter peaks with low template/n-2 stutter contributing, that are below the laboratory stutter threshold):

DNA WI-34 Analysis Workflow for Questioned Samples

Document ID: 5159
Revision: 10

Effective Date: 3/9/2022

Status: Retired Page 2 of 7

 If you clicked off a stutter peak because pull-up contributed to it, leave this peak in your STRmix project, provided that the peak is still greater than AT, with pull-up subtracted,

o If you clicked off a peak as high stutter, leave this peak in your STRmix project.

and the pull-up is not responsible for the majority of the peak height.

- O Same goes if any of these peaks are a combo of different stutters
 - There might be exceptions to this, for example, if the subtraction of pull-up brings the stutter peak below AT, STRmix should not see this peak, and if the pull-up contribution adds an unusually large amount of height to the stutter peak, it might be better for STRmix not to see such a high peak. Ask for assistance if you are unsure.
 - Peaks that you clicked off with n-2 stutter contributing, that are at or below the laboratory's stutter threshold, will remain in the STRmix project. These will not be listed as non-concordances when running the project comparison.
- 14) Re-save GeneMarker file when all edits have been made and all samples have been disabled.
- 15) Export report as a text file, same location as file created in step 3 on page 1.
- 16) Create DNA-QR-302 Contributor Estimation Worksheet for each sample with results. If it's a single source sample, or you feel you don't need the macro to assist you in determining number of contributors, you still need this sheet, but add notes to the comments area as to your determination of number of contributors.
 - o Minimum number of male contributors will auto-populate on designated line.
- 17) Initial and date QR-4A (Batch Paperwork Review Worksheet).
- 18) Give batch to Technical Reviewer.

Technical Reviewer

1) Review all batch paperwork.

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- 2) Open the analyst's fully analyzed allele table project.
- 3) Review samples, note any differences to the 1st Analyst. Check that:
 - a. Correct analysis template is used.
 - b. Analyst's assessment of size standards, ladders and controls are appropriate.
 - c. All called peaks appear to be true alleles.
 - d. All deletions/edits made are appropriate.
 - e. The project comparison QR has appropriate number of non-concordances.
 - f. You are in agreement with the number of contributor estimation.
- 4) Document any corrections on QR-347.
- 5) Initial and date OR-4A.
- 6) Give batch to Administrative Reviewer.

Administrative Reviewer

1) Review all batch paperwork.

DNA WI-34 Analysis Workflow for Questioned Samples	Document ID: 5159
	Revision: 10
	ECC /: D / 2/0/2022

Effective Date: 3/9/2022

Status: Retired Page **3** of **7**

2) Document any corrections on QR-347.

3) Initial and date QR-4A.

4) Give batch back to Analyst.

Approved by Director: Dr. Guy Vallaro

Analyst

- 1) Make changes to project(s), if necessary, according to the technical review. Analytical changes will require updates to allele table GeneMarker project & report text file, STRmix GeneMarker project & report text file and number of contributors worksheet.
- 2) Complete batch cross comparison and staff search ensuring all completed injections are included.
- 3) Run DNA-QR-301 Project Comparison Tool using the exported text files for each fully analyzed project and associated STRmix project.
 - a. If there are any discrepancies not accounted for in your analysis, you must correct them in the appropriate GeneMarker project. Then re-save the GeneMarker projects AND re-export the text files AND re-run project comparison macro.
 - b. Keep repeating this until the number of discrepancies can be explained by your analysis.
 - c. Print out this sheet for the batch paperwork; you only need the final copy in your batch.
- 4) Complete QR-347. Initial and date QR-4a. Return batch to Technical Reviewer.

Technical Reviewer

- 1) Review any changes made by 1st analyst and new QRs (batch cross contamination and project comparison tool)
- 2) Add batch name to spreadsheet on U: Drive.
- 3) Initial and date QR-4A.
- 4) Return batch to Analyst.

Analyst

- 1) Scan batch paperwork (not containing sample e-grams & contributor estimator sheets) & save PDF file to U: Drive.
- 2) Move HID and project files from "To Be Analyzed" to "Completed" folder on U: Drive.
- 3) Give batch paperwork to Technical Reviewer to review.
- 4) Each case jacket will contain original e-grams and contributor estimator worksheets and photocopies of QR-4a (Batch Paperwork Review QA Checklist), QR-346 (Male Screen Results Worksheet) and QR-48 (Halt at Quant List), as appropriate.

Technical Reviewer

- 1) Ensure all pages scanned and PDF file on U: Drive is complete and accurate.
- 2) Ensure HID folders have been moved to U:\3130 NUCLEAR CASEWORK & QC\Completed.
- 3) Add initials to batch paperwork spreadsheet.

DNA WI-34 Analysis Workflow for Questioned Samples

Document ID: 5159

Revision: 10

Effective Date: 3/9/2022

Status: Retired Page 4 of 7

CASEWORK

Analyst

1) Enter all possible profiles into CODIS.

Approved by Director: Dr. Guy Vallaro

- 2) Make comparisons of all knowns to all Q's in case.
 - a. Does a sample have 5+ contributors?
 - i. If so, do any of the knowns CBE to the sample warrant being conditioned? If yes, does that bring it down to 4 unconditioned contributors?
 - 1. Yes can interpret.
 - 2. No Clear major present can make comparison to deduced major profile. No comparisons will be made to minor components of profile.
 - 3. No No clear major present No comparisons will be made to profile.
 - b. Samples that are clear eliminations are not run through STRmix, "Eliminate by Analyst" in report macro.
 - c. Comparisons that are not clear eliminations, are run through STRmix.
 - i. Reported out comparison based on LR calculated.
 - ii. The "Sample ID" field shall contain both item # and known # (if applicable), so that there is a unique identifier in the footer of all pages when printed.
 - d. If reporting out a statistic for a comparison, deconvolute in STRmix with appropriate knowns conditioned to profile.
 - i. Report out comparison based on LR calculated.
 - ii. If not reporting out a statistic (i.e. intimate sample with no suspect known submitted, single source sample when known expected on sample), no need to run STRmix, enter "assumed" in report workbook. STRmix may be run if necessary to determine if assuming is appropriate.
 - e. Does a sample only have one allele at one locus? → inconclusive or elimination by analyst
 - f. Are you unable to determine the number of contributors with reasonable scientific certainty?
 - i. Clear major present Can make comparison to deduced major profile. No comparisons will be made to minor components of profile.
 - ii. No clear major present No comparisons will be made to profile.
 - g. Is there a problem with the deconvolution discovered Re: 2nd diagnostics that can't be resolved? → inconclusive or elimination by analyst
- 3) If a sample is deconvoluted through STRmix:
 - a. The file will automatically save onto the server, under F:\results.
 - b. Review the summary report. Ensure that the input file is correct. The letter "S" has previously been added to the beginning of the sample names of the STRmix input file when the project comparison macro was run.

DNA WI-34 Analysis Workflow for Questioned Samples Document ID: 5159 Revision: 10 Effective Date: 3/9/2022 Approved by Director: Dr. Guy Vallaro Status: Retired

Status: Retired Page 5 of 7

c. Print out pages 1 thru 3 of the STRmix report, or more if "Per Locus Likelihood Ratios" for the three reported populations groups is on subsequent pages.

- d. Scrutinize summary reports for any problems with the deconvolution.
 - i. Fill out DNA-QR-303 for the 2nd diagnostics for each deconvolution. Any assumed contributors will be recorded on DNA-QR-303.
- 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-XXXXX, and move that folder into this newly created case specific folder.
- 4) Review case jacket, write report.
- 5) Give case jacket to TR.

Technical Reviewer

- 1) Review case jacket, comparisons and report as you normally would. Document any corrections on QR-347.
 - a. If you disagree with an eliminated comparison, the 1st Analyst will have to run this sample through STRmix.
 - b. Review the summary report. Ensure that the input file is correct. The letter "S" has previously been added to the beginning of the sample names of the STRmix input file when the project comparison macro was run.
- 2) If you need to review more of the summary 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 to AR. If a separate AR is not required, complete administrative review tasks as well.

Administrative Reviewer

- 1) Review case jacket and report. Document any corrections on QR-347.
- 2) Give case jacket back to Analyst.

Analyst

- 1) Make all appropriate corrections to report and case jacket and document on QR-347.
- 2) If the TR disagreed with one of your elimination calls, you must run that sample through STRmix, print out appropriate pages and fill out DNA-QR-303.
 - a. The report will have to be re-worked:
 - i. If it is still an elimination, it is now "elimination by STRmix", and not "elimination by analyst", report wording will be different.
 - 1. If it is no longer an elimination, report out result based on LR.
- 3) Once report corrections are complete, save the draft report as both a Word Document and PDF to U:Casereports. Electronically sign the PDF using a secure certificate and save to U:Casereports.

DNA WI-34 Analysis Workflow for Questioned Samples Document ID: 5159

Revision: 10

Effective Date: 3/9/2022

Status: Retired Page 6 of 7

Approved by Director: Dr. Guy Vallaro

If there are STRmix results, move the folder for this case from your folder on the server's F: Drive to F:\results\Completed.

4) Give case jacket to TR for correction check.

Technical Reviewer

- 1) Ensure all corrections are accurate.
- 2) If additional deconvolution work needed to be completed, make sure new wording in report is accurate and review all additional paperwork.
- 3) If there's STRmix data, check to make sure the folder has been moved to F;\results\Completed.
- 4) Electronically sign the report PDF using a secure certificate and save the final report to U:Casereports.
- 5) Give completed case jacket to analyst...

CODIS

Analyst

- 1) If there is a CODIS hit for a sample of yours, you will be notified by the CODIS administrator/alternate or their designee.
- 2) The CODIS hit might warrant the sample to be deconvoluted in STRmix. If a deconvolution of this sample has already been completed, the case jacket can be brought to the CODIS administrator/alternate or their designee.
- 3) If a deconvolution of this sample has not yet been completed, deconvolute the sample, making sure to condition any knowns that you assumed when writing the report.
 - a. Print out page 1 of the STRmix report, fill out DNA-QR-303, and give the case jacket to your TR to review the additional paperwork. You can check, initial, and date next to 3.D. on DNA-QR-4.

Technical Reviewer

- 1) Review additional paperwork in case jacket.
- 2) Check, initial and date next to 3.D. on DNA-QR-4.
- 3) Give the case jacket to the AR

Analyst

1) When you receive your case jacket back for initials, move your STRmix folder to F:\results\completed. Check, initial, and date that you did so on DNA-QR-4.

OTHER

1) If a known comes in later on that warrants being conditioned, all deconvolutions need to be repeated with a new deconvolution, and a new statistic is calculated.

DNA WI-34 Analysis Workflow for Questioned Samples

Document ID: 5159

Revision: 10

Effective Date: 3/9/2022

Status: Retired Page 7 of 7

Approved by Director: Dr. Guy Vallaro

- 2) If a known comes in later that's not clearly eliminated regarding a sample that has already been deconvoluted, use the "LR to previously analysis" function of STRmix, and do not repeat the deconvolution.
- 3) Similarly, if you have a Q in the case, and two knowns are CBE to that sample, only deconvolute the sample once. For the 2nd known, use the "LR to previous analysis" function of STRmix.

