PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 1 OF 11

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1 Purpose

1.1 To compile the sequence data generated by the MiSeq/MiSeq FGx and Verogen Universal Analysis Software (UAS) into a project for analysis, by editing the sequence data and compiling a consensus sequence that can be compared with the revised Cambridge Reference Sequence (rCRS) to determine the mitochondrial DNA type.
Procedure

1.2 The analysis will use two elements of software: Qiagen CLC Genomics Workbench, with the AFDIL-Qiagen mtDNA Expert (AQME) toolkit plugin.

1.3 The data following the MiSeq run will be saved on the PowerWulf server, in individual folders of FASTQ files for analysis. The files will also be stored in an appropriate folder in M:\MPS_Data\.

1.4 The files with "R1" filenames will be imported into the CLC software for analysis, using the program's "Promega NGS mito workflow". The R1 files will be trimmed and aligned, with variant tables output in excel tab-delimited format.

2 Import data for processing

2.1 Open the Qiagen CLC Genomics Workbench from your computer's start menu.

2.1.1 If the software does not appear to be installed, submit a HelpDesk ticket for installation.

**PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS**

Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 2 OF 11

2.1.2   If the following message appears, then another user is currently using the department's network license.
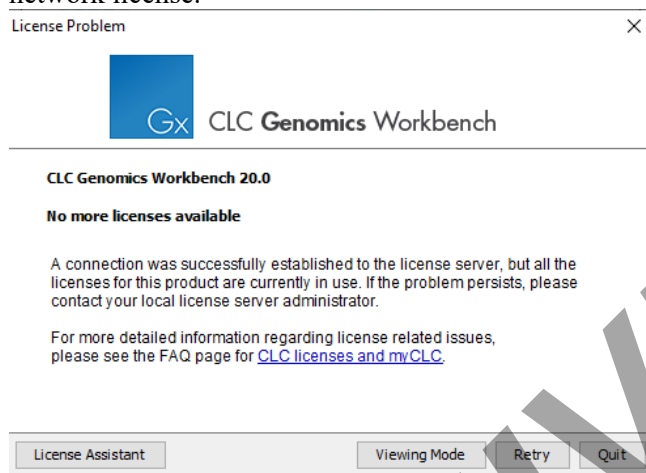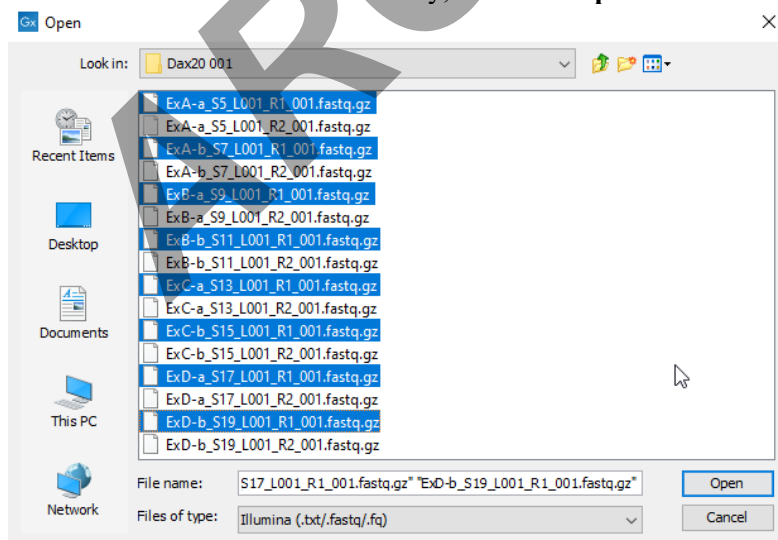

2.2   Go to File -> Import -> Illumina.

2.3   Navigate to the PowerWulf subdirectory that contains the FASTQ files for analysis.

2.3.1   If the PowerWulf server is not mapped to a network drive on your computer, please contact a supervisor for the IP address and login credentials.

2.4   Select the R1 files from the directory, and click **Open**.

2.5   Confirm the selected R1 files are listed in the subsequent screen. Click **Next.**

**PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS**

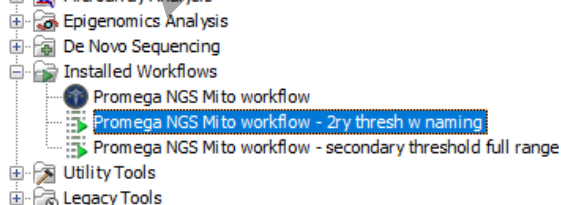| Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit	
Status: Published	Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 3 OF 11

2.6 Under **Result handling**, choose the **Save** radio button, and be sure that **Create subfolders per batch unit** is NOT checked, then click **Next.**

2.7 On the directory navigation screen, navigate to the casework folder and click **New Folder** to create a subdirectory for your run. Enter the run name as the name of the new folder, then click **OK**.

2.8 Click **Finish**.

2.9 Confirm that the selected files are now present in the Navigation Area in the top left of your screen.
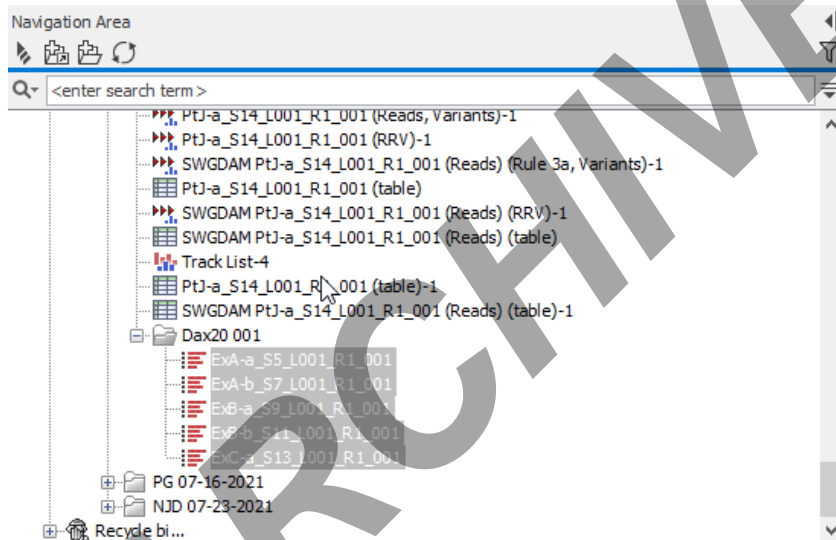
3 Run the analysis workflow

3.1 In the Toolbox -> Toolbox menu, click the + to expanded the Installed Workflows items, and double-click on "Promega NGS Mito workflow – 2ry thresh w naming".

3.1.1 ==If this workflow is not installed, you may find the installer in the M:\MPS_Data\ directory. The current file is "Promega NGS Mito workflow – 2ry thresh w naming-0.1.cpw"==

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 4 OF 11

3.2   If your R1 files are not already present in the Selected Elements area on the right of the screen, click to highlight your R1 files and click the **Right Arrow** to add them to the Selected Elements.
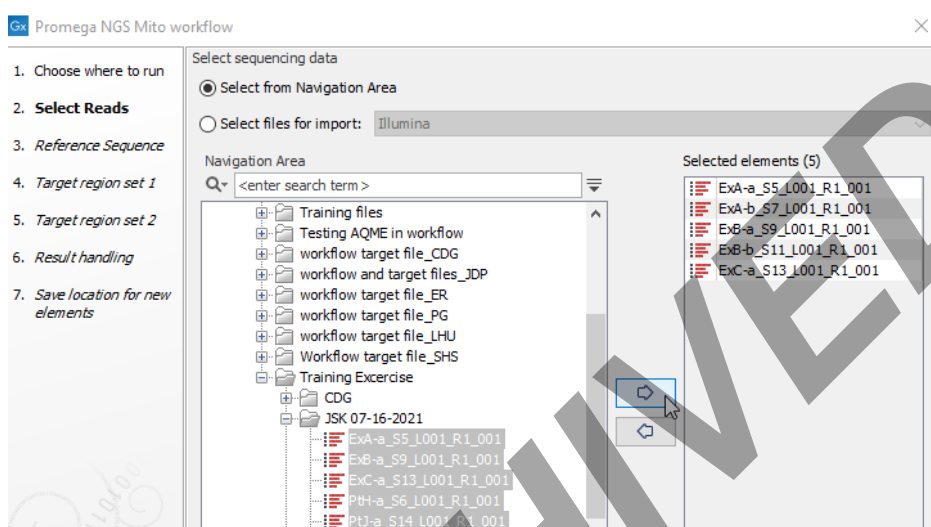
3.3   Check the **Batch** button under the Navigation area, then click **Next**.

3.4   For Configure batching, the **Use organization of input data** radio box should be selected. Click **Next**.

3.5   For Batch overview, the selected R1 files should be in the Units list on the left. Click **Next**.

3.6   For Workflow Input, confirm that **NC_012920 (Genome)** is selected by default.

3.6.1   If it is not selected, click on the icon and select **NC_012920 (Genome)** from the CLC_Data/Workflow files in the Navigation Area on the left side, click the **Right arrow** to add to the Selected Elements, and click **OK**.
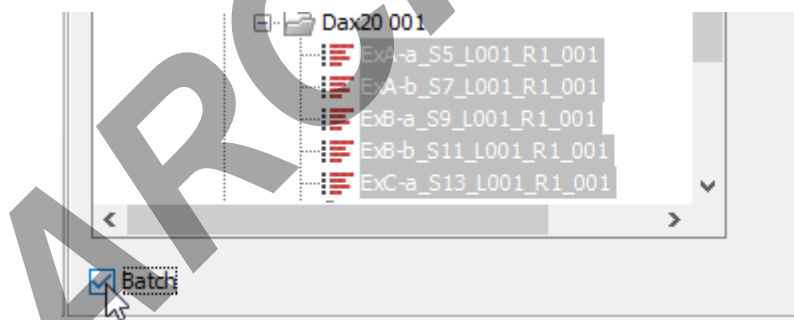
PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 5 OF 11

3.7   Click **Next**.

3.8   For Target region set 1, confirm that NC_012920 (Genome) selection set 1 -600 threshold (Misc. feature) is selected by default.

    3.8.1   If it is not selected, click on the 🔍 icon and select **NC_012920 (Genome) selection set 1 -600 threshold (Misc. feature)** from the CLC_Data/Workflow files in the Navigation Area on the left side, click the **Right arrow** to add to the Selected Elements, and click **OK**.

3.9   Click **Next**.

3.10   For Target region set 2, confirm that **NC_012920 (Genome) selection set 1 -600 threshold (Misc. feature)** is selected again by default.

    3.10.1   If it is not selected, click on the 🔍 icon and select **NC_012920 (Genome) selection set 1 -600 threshold (Misc. feature)** from the CLC_Data/Workflow files in the Navigation Area on the left side, click the **Right arrow** to add to the Selected Elements, and click **OK**.
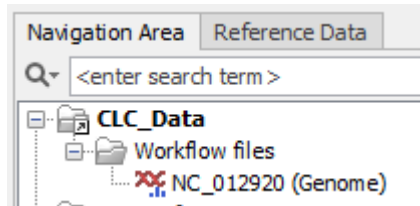
3.11   Click **Next**.

3.12   For Result handling, check the **Save** radio button, and check **Create workflow result metadata**. Click **Next**.

3.13   For Save location for new elements, select the run folder in the navigation area and click **Finish**.

    3.13.1   The analysis progress can be seen in real time by clicked the Processes tab in the Toolbox, on lower left side of the screen.

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 6 OF 11
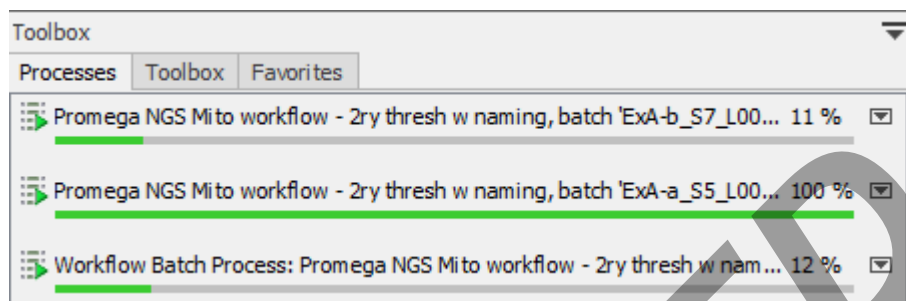
4 Exporting results

4.1 In the Navigation area, right-click on the folder name of your project, and select **Sort folder**.
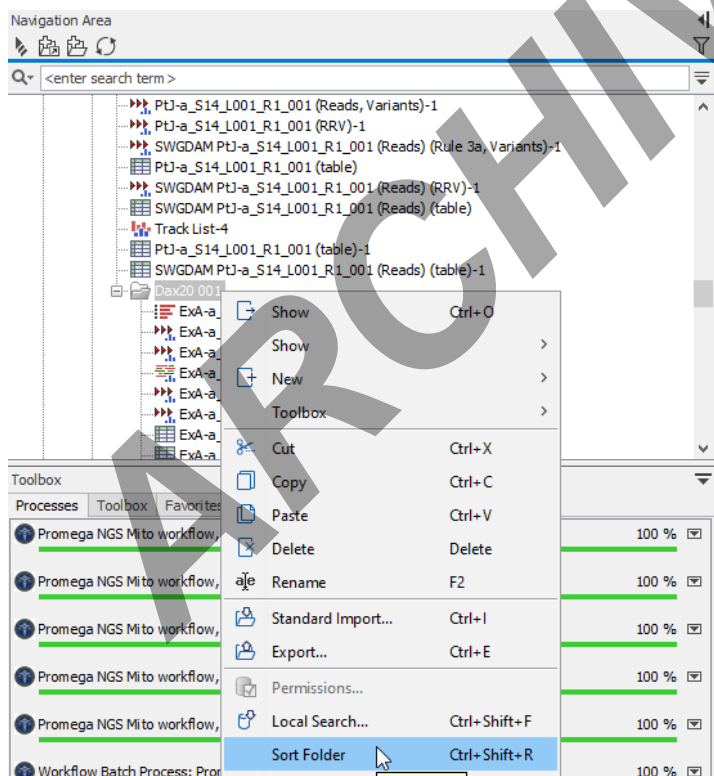
4.2 Scroll down the output file list, until you reach the files beginning with **SWGDAM**. Click and select all SWGDAM files with the variant table icon ▦ . Each sample should have two tables whose names end in "-primary" and "-secondary", respectively. These correspond with the 600-read and 100-read analysis thresholds respectively.

	Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit	
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 7 OF 11

4.3   With the files selected, click the **Export** icon along the top menu.

4.4   Select the output format for the tables. Select **Excel 97-2007** and click the **Select** button.

4.5   Confirm there are two SWGDAM files for each template library in the Selected Elements table on the right side of the screen, one for each of the two analysis thresholds used to analyze each library.

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

| Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 8 OF 11

4.5.1 If any are absent from the Selected elements list, locate the missing file(s) in the Navigation area, click to select, and click the **right arrow** to populate them into the Selected elements list.

4.6 Check the **Batch** button under the Navigation area, then click **Next**.

4.7 Under Batch overview, the selected pairs of variant table, with names ending in **(table)** and **(table)-1** should be present in the Units box.

4.8 Under Specify export parameters, Output as a single file should NOT be checked. Export all columns. (Deselect to specify columns in next step) SHOULD be checked. Click Next.

4.9 Under Select output folder, navigate to the desired directory and click **Finish**.

5 Processing the files for import into LIMS

5.1.1 The exported Excel filenames follow the following default naming structure:

5.1.2

5.1.3 SWGDAM FB00-00000-1-1-1-1_samplename-suffix-[well#]_[sample#]_L001_R1_001 (Reads) (table)-secondary.xlsx

5.1.4

5.1.5 Example: SWGDAM FB20-00000-1-1-1-bone5-UHR-reamp-F3_S23_L001_R1_001 (Reads) (table)-1-secondary.xlsx

5.1.6 The filename of the text file needs to be modified for successful import in LIMS. This can be accomplished by either copying/pasting from LIMS (5.1.2 below) or manually changing the filename (5.1.3 below)

5.1.7 If changing the filename by copying/pasting from LIMS, copy the full sample name and replace the text between ("SWGDAM " and "(Reads)") with the copied text.

Example:

Paste copied text over the italicized portion of the filename, between the two bolded

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

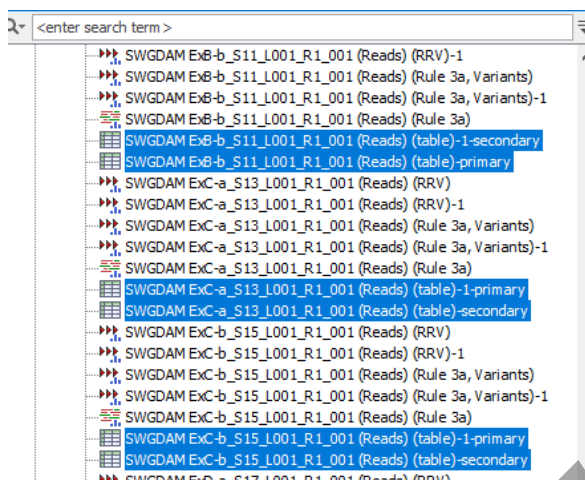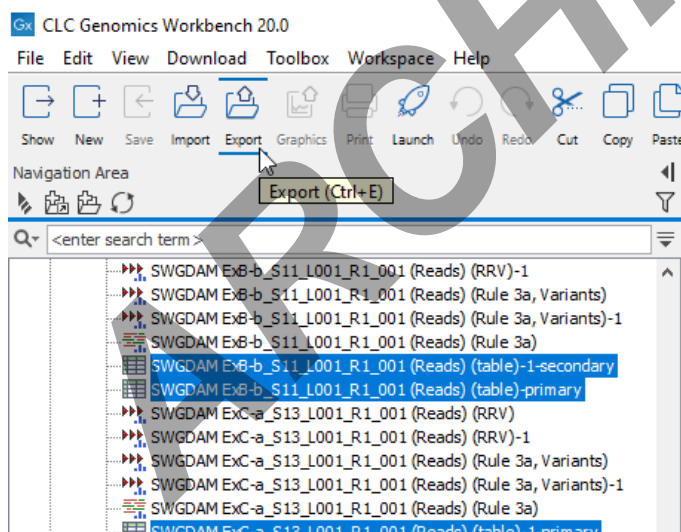|		
Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 9 OF 11

sections below:

SWGDAM *FBPT22-00014-22524-1-1-vic-BL-reamp-F4_S24_L001_R1_001* **(Reads)** (table)-secondary.xlsx

becomes

SWGDAM *FBPT22-00014_22524_1.1_vic_BL_reamp_F4* **(Reads)** (table)-secondary.xlsx

5.1.8   If changing the filename manually, make the following changes.

5.1.8.1   Compare the text in the filename between "SWGDAM " and "sample#" to the corresponding sample name in the LIMS test batch. Change any hyphens to periods, spaces and/or underscores to make that section of the filename match the sample name is LIMS.

Example: SWGDAM FB20-00000-1-1-1-bone5-UHR-reamp-F3_S23_L001_R1_001 (Reads) (table)-1-secondary).xlsx

becomes…

SWGDAM FB20-00000_1_1.1_bone5_UHR_reamp_F3_S23_L001_R1_001 (Reads) (table)-1-secondary).xlsx

5.1.8.2   Delete "[sample#]_L001_R1_001 " from the right side of the filename.

Example: SWGDAM FB20-00000_1_1.1_bone5_UHR_reamp_F3~~_S23_L001_R1_001~~ (Reads) (table)-1-secondary).xlsx

becomes

SWGDAM FB20-00000_1_1.1_bone5_UHR_reamp_F3 (Reads) (table)-1-secondary).xlsx

5.1.8.3   The section of the filename between "SWGDAM " and " (Reads)" should be the same as the LIMS sample name in the test batch.

5.1.9   Open the renamed excel file, select the "mtDNA table" tab, go to "File", "Save as", and choose "Text (tab-limited) (*.txt)" as the file type. Save the text file in the appropriate data directory.

5.1.10   Any text files that are empty due to no data in the "mtDNA table" tab of the excel file, will need to have a header row added to it.

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit		
Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 10 OF 11

5.1.10.1 Open a text file that is not empty, such as a positive control

5.1.10.2 Copy the first line of the text file, starting with "Position"

5.1.10.3 Paste it into the empty text file and save.

6 Importing the text files into LIMS

6.1 Select your test batch in LIMS, and click the "Edit" button

6.2 Under "Plate/Analysis Set", check the checkbox next to the plate name and click "Data Entry"

6.3 From the menu options at the top of the screen, select "MPS Import"

6.4 Select the appropriate data and import into the batch

6.5 Check the samples in data entry for insertions/deletions. Wherever there are insertions/deletions, please enter ":" in the appropriate empty "Variant" and "Ref Sequence Value" cells as appropriate.

315.1	:	C	C (97.9%)
498	C	:	-0.989
499	G	A	A (99.2%)

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit

Status: Published		Document ID: 50912
DATE EFFECTIVE 06/02/2022	APPROVED BY mtDNA Technical Leader	PAGE 11 OF 11

6.6 Check the samples in data entry where there are no differences from the reference sequence. In those instances, LIMS have have an empty cell under "Variant." If so, you will need to manually add a "-" (hyphen) into those empty cells to finalize the batch.

A	Primary		-	-	-		-

6.7 Review all controls. If there are negative controls that have covered bases, note in the test batch comments the highest read count for those controls.

Batch Configuration	MiSeqSequencing	Test Batch Type	Case
Notes	ENeg2-08302021-082555-A2 – 197 covered bases (highest coverage 152 reads) ENeg2-09022021-074549-C1 – 2 covered bases (highest coverage 100 reads) ENeg2-12222021-084216-B4 – 195 covered bases (highest coverage 221 reads) ENeg3-05132021-115643-H3 – 47 covered bases (highest coverage 138 reads) ENeg3-10072021-141930-C3 – 210 covered bases (highest coverage 315 reads)		
Functional Group	mtDNA	Method Analysis	