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

Status: Published DATE EFFECTIVE

APPROVED BY mtDNA Technical Leader

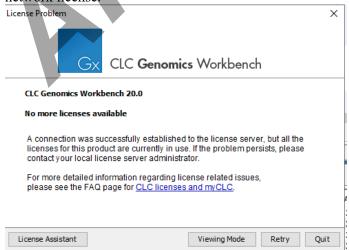
Mitochondrial DNA Massively Parallel Sequencing (mitoMPS) Analysis using the Qiagen CLC Workbench and AQME Toolkit

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 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.

Import data for processing 2

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



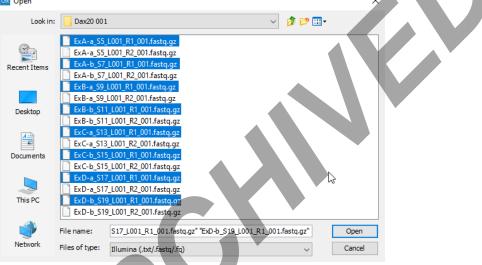
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2.2 Go to **File -> Import -> Illumina**.

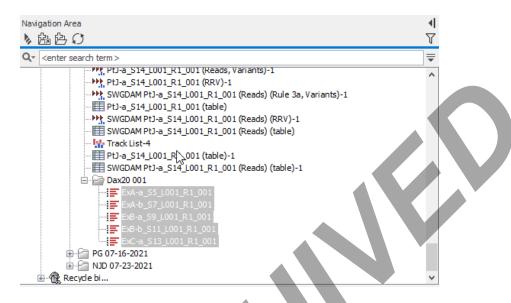
- 2.3 Navigate to the PowerWulf subdirectory that contains the FASTQ files for analysis.
 - 2.3.1 If the PowerWulf server is not mapped to a network drive on your computer, please contact a supervisor for the IP address and login credentials.
- 2.4 Select the R1 files from the directory, and click **Open**.



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

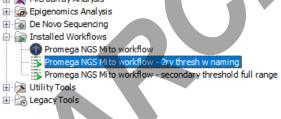
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3 Run the analysis workflow

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".

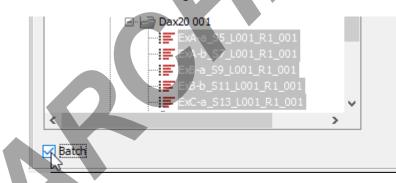


3.2 If your R1 files are not already present in the Selected Elements area on the right of the screen, click to highlight your R1 files and click the **Right Arrow** to add them to the Selected Elements.

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	Promega NGS Mito wo Choose where to run Select Reads	 Select from Navigation Area 						
	3. Reference Sequence	O Select files for import: Illumina		Selected elements (5)				
	4. Target region set 1	Q+ <enter search="" term=""></enter>	—	ExA-a S5 L001 R1	001			
	5. Target region set 2 6. Result handling	Training files Training files Testing AQME in workflow Or resting AQME in conduction	^	ExA-b_S7_L001_R1	_001 1_001			
	7. Save location for new elements	 workflow and target files_JDP workflow target file_ER workflow target file_PG workflow target file_LHU Workflow target file_SHS Training Excercise CDG 		i; ExC-a_S13_L001_R	1_011			

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

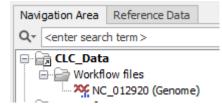
□ → JSK 07-16-2021 → ■ ExA-a_S5_L → ■ ExA-a_S9_L → ■ ExB-a_S9_L → ■ ExC-a_S13_ → ■ PtH-a_S6_L → ■ Pth-a_S14



- 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.

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- 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 $\stackrel{[m]}{\longrightarrow}$ 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 $\stackrel{\frown}{\sim}$ 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 Reult 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.

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Toolbox				-
Processes	Toolbox	Favorites		
Fromeg	a NGS Mito	workflow - 2	ry thresh w naming, batch 'ExA-b_S7_L00 11 %	
Promeg	a NGS Mito	workflow - 2	ry thresh w naming, batch 'ExA-a_S5_L00 100 %	
🚯 Workflo	w Batch Pro	ocess: Prome	ga NGS Mito workflow - 2ry thresh w nam 12 %	

4 Exporting results

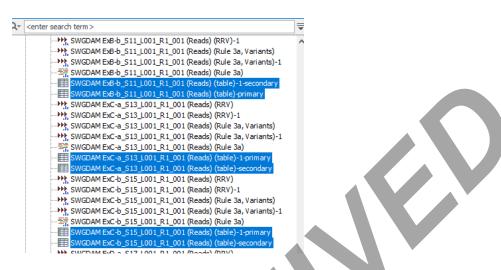
4.1 In the Navigation area, right-click on the folder name of your project, and select **Sort folder**.

Navigation Area			II III
▶協臣♀			V
Q. ✓ <enter search="" term=""></enter>			₹
→ PU-a_S14_L0 → SWGDAM PU-a_S14_L0 ■ PU-a_S14_L0 → SWGDAM PU-a_S14_L0 → SWGDAM PU-a_S14_L0 → Track List-4 → BVGAM PU-a_S14_L0 → SWGDAM PU-a_S14_L0 → SWGDAM PU-a_S14_L0 → SWGAM PU-a_S14_L0	a_S14_L001_R1_001 (Read a_S14_L001_R1_001 (Read 01_R1_001 (table)-1 a_S14_L001_R1_001 (Read	is) (Rule 3a, Variants)-1 is) (RRV)-1 is) (table)	
ExA-a ExA-a ExA-a	+ New Toplbox	> Ctrl+X	
Toolbox	Сору	Ctrl+C	T
Processes Toolbox Favorites	Paste	Ctrl+V	
Promega NGS Mito workflow,	Delete	Delete	100 % 💌
😚 Promega NGS Mito workflow,	aje Rename	F2	100 % 💌
Promega NGS Mito workflow,	Standard Import	Ctrl+I	100 % 💌
	🕒 Export	Ctrl+E	
Promega NGS Mito workflow,	Permissions		100 % 💌
Promega NGS Mito workflow,	🖞 Local Search	Ctrl+Shift+F	100 % 💌
Workflow Batch Process: Pror	Sort Folder 🛛 🔓	Ctrl+Shift+R	100 % 💌

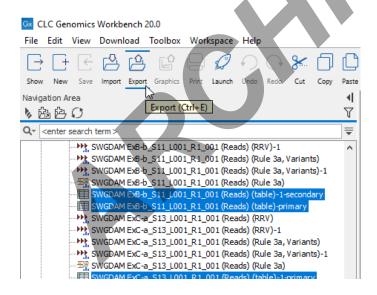
4.2 Scroll down the output file list, until you reach the files beginning with **SWGDAM**. Click and select all SWGDAM files with the variant table icon ^{IIII}. Each sample should have two tables whose names end in "-primary" and "-secondary", respectively. These correspond with the 600-read and 100-read analysis thresholds respectively.

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



- 4.4 Select the output format for the tables. Select **Tab-delimited text** and click the **Select** button. These will be imported into the LIMS MiSeq test batch via Data Entry.
- 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.

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- 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**.
- 4.10 Click the **Export** button again, and this time select **Excel 97-2007** and click the **Select** button. Repeat steps 4.5-4.9.
- 4.11 Import the excel files into the MiSeq test batch in the Data Entry screen