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

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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.

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

License Problem	×
Gx CLC Genomics Workbench	
CLC Genomics Workbench 20.0	
No more licenses available	
A connection was successfully established to the license server, but all the licenses for this product are currently in use. If the problem persists, please contact your local license server administrator.	
For more detailed information regarding license related issues, please see the FAQ page for <u>CLC licenses and mrCLC</u> .	
License Assistant Viewing Mode Retry Q	uit

2.2 Go to File -> Import -> Illumina and ensure that the settings are as below:

🐼 Illumina High-Throughput	Sequencing Import X
1. Choose where to run	Select files of types Illumina (.txt/.fastq/.fq) Selected Files (0)
2. Import files and options 3. Result bandling	
4. Save location for new	
elements	Select files or folders to import with the buttons below
	Add folders Add files Remove
	General options Image: Paired reads Paired reads Paired read information Discard read names O Paired-end (forward-reverse) Minimum distance Maximum distance Minimum distance 1
100 110 10 100 110 10	Illumina options Illumina options Remove failed reads Quality scores NCBI/Sanger or Illumina Pipeline 1.8 and later > MiSeq de-multiplexing Trim reads Join reads from different lanes >
Help Reset	Previous Next Finish Cancel

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- 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** sample files from the directory, and click **Open**.

Gx Open		×
Look in:	🔁 Dax20 001 🗸 🤌 🔁	
Recent Items	ExA-a_S5_L001_R1_001.fastq.gz ExA-a_S5_L001_R2_001.fastq.gz ExA-b_S7_L001_R1_001.fastq.gz ExA-b_S7_L001_R2_001.fastq.gz ExA-b_S7_L001_R2_001.fastq.gz ExA-b_S7_L001_R2_001.fastq.gz	
Desktop	ExB-a_S9_L001_R2_001.fastq.gz ExB-b_S11_L001_R1_001.fastq.gz ExB-b_S11_L001_R2_001.fastq.gz ExC-a_S13_L001_R1_001.fastq.gz	
Documents	ExC-a_S13_L001_R2_001.fastq.gz ExC-b_S15_L001_R1_001.fastq.gz ExC-b_S15_L001_R2_001.fastq.gz ExC-b_S15_L001_R2_001.fastq.gz	
This PC	ExD-a_S17_L001_R2_001.fastq.gz ExD-b_S19_L001_R1_001.fastq.gz ExD-b_S19_L001_R2_001.fastq.gz	μ <u>ς</u>
Network	File name: S17_L001_R1_001.fastq.gz* "ExD-b_S19_L001_R1_001.fastq.gz* Files of type: Illumina (.txt/.fastq/.fq)	Open Cancel

- 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".



- 3.1.1 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"
- 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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Gx	Promega NGS Mito we	orkflow				\times
1.	Choose where to run	Select sequencing data				
2.	Select Reads	Select files for import: Illumina				~
3.	Reference Sequence	Navigation Area		Sele	cted elements (5)	
4.	Target region set 1	Q < <enter search="" term=""></enter>	₹	15	ExA-a_S5_L001_R1_001	
5.	Target region set 2	⊕ · · · · · · · · · · · · · · · · · · ·	^		ExA-b_S7_L001_R1_001 ExB-a_S9_L001_R1_001	
6.	Result handling	workflow target file_CDG workflow and target files_JDP			ExB-b_S11_L001_R1_001 ExC-a_S13_L001_R1_001	
7.	Save location for new elements		İ			
	000	□ □				

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.

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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 ^[1] 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 ^[X] 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 in specified location** radio button, and check **Create workflow result metadata**. Click **Next**. (If the radio button only says "Save", use the "Previous" button to go back to the "Select Reads" step and make sure that the "Batch" box is checked off.)
- 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		
Promeo	a NGS Mito	workflow - 2	ry thresh w paming batch 'ExA-b S7 100 11 %	
		monthion 2	ry uncarrwindning, batch EXR-0_37_c00 11 /6	Ľ
			y direan whanning, batch EXA 0_37_000 11 /	Ľ
Promeg	a NGS Mito	workflow - 2	ry thresh w naming, batch 'ExA-a_S5_L00 100 %	
Promeg	a NGS Mito	workflow - 2	ry thresh w naming, batch 'ExA-a_S5_L00 11 %	

Exporting results 4

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

Navigation Area			•			
>協会 ()			T			
Q- <enter search="" term=""></enter>			₹			
PtJ-a_S14_L	001_R1_001 (Reads, Varian	ts)-1	^			
PtJ-a_S14_L	001_R1_001 (RRV)-1					
SWGDAM PtJ-a_S14_L001_R1_001 (Reads) (Rule 3a, Variants)-1						
PtJ-a_S14_L001_R1_001 (table)						
SWGDAM Pt	J-a_S14_L001_R1_001 (Rea	ds) (RRV)-1				
📰 SWGDAM Pt	J-a_S14_L001_R1_001 (Rea	ds) (table)				
👫 Track List-4						
PtJ-a_S14_L	001_R1_001 (table)-1					
SWGDAM Pt	J-a_S14_L001_R1_001 (Rea	ds) (table)-1				
🖻 🗁 Dax20 001						
ExA-a_	L→ Show	Ctrl+O				
ExA-a	Show	>				
ExA-a						
EXA-a	L+ New	>				
EXA-a	Toolbox	>				
EvA-a	100100x					
ExA-a	⊱ Cut	Ctrl+X	~			
Toolbox	Сору	Ctrl+C	•			
Processes Toolbox Favorites	Paste	Ctrl+V				
Promega NGS Mito workflow,			100 % 💌			
	Delete	Delete				
Promega NGS Mito workflow,	ale Rename	F2	100 % 💌			
	🖄 Standard Import	Ctrl+I	100.00			
Promega NGS Mito workflow,	r0. Europh	OH F	100 %			
-	🗁 Ехроп	Ctri+E				
Promega NGS Mito workflow,	Permissions		100 % 💌			
Promega NGS Mito workflow,	🕑 Local Search	Ctrl+Shift+F	100 % 💌			
	Sort Folder	Ctrl+Shift+R				
Morkflow Batch Process: Pror	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		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 🕮. Each sample should have two tables whose names end in "-primary" and "-secondary", respectively. These correspond with the 600read and 100-read analysis thresholds respectively.

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ک ۔	<enter search="" term=""></enter>	-
_	SWGDAM ExB-b_S11_L001_R1_001 (Reads) (RRV)-1	1
	SWGDAM Ex8-b_S11_L001_R1_001 (Reads) (Rule 3a, Variants)	
	SWGDAM ExB-b_S11_L001_R1_001 (Reads) (Rule 3a, Variants)-1	
	=== SWGDAM ExB-b_S11_L001_R1_001 (Reads) (Rule 3a)	
	SWGDAM Ex8-b_S11_L001_R1_001 (Reads) (table)-1-secondary	
	SWGDAM Ex8-b_S11_L001_R1_001 (Reads) (table)-primary	
	SWGDAM ExC-a_S13_L001_R1_001 (Reads) (RRV)	
	SWGDAM ExC-a S13 L001 R1 001 (Reads) (RRV)-1	
	SWGDAM ExC-a S13 L001 R1 001 (Reads) (Rule 3a, Variants)	
	SWGDAM ExC-a S13 L001 R1 001 (Reads) (Rule 3a, Variants)-1	
	SWGDAM ExC-a S13 L001 R1 001 (Reads) (table)-1-primary	
	SWGDAM ExC-a S13 L001 R1 001 (Reads) (table)-secondary	
	SWGDAM ExC-b S15 L001 R1 001 (Reads) (RRV)	
	SWGDAM ExC-b S15 L001 R1 001 (Reads) (RRV)-1	
	SWGDAM ExC-b S15 L001 R1 001 (Reads) (Rule 3a, Variants)	
	SWGDAM ExC-b S15 L001 R1 001 (Reads) (Rule 3a, Variants)-1	
	SWGDAM ExC-b S15 L001 R1 001 (Reads) (Rule 3a)	
	SWGDAM ExC-b S15 L001 R1 001 (Reads) (table)-1-primary	
	SWGDAM ExC-b_S15 L001 R1_001 (Reads) (table)-secondary	
	COMPLEX D = 517 L001 D1 (Codd) (DDV)	

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.

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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**.

5 Importing Control data into LIMS

- 5.1 Negative Controls
 - 5.1.1 For each amplification negative control and extraction negative control, open the primary threshold analysis file for each sample, which has a filename ending in "-primary".
 - 5.1.2 Click on the "Report" tab. Print/save the "Report" tab to a PDF file
 - 5.1.3 Upload all negative control PDFs to the "Attachments" tab of the MiSeq test batch.
- 5.2 Positive controls
 - 5.2.1 Open the secondary threshold analysis file for the positive control, which has a filename ending in "-secondary".
 - 5.2.2 Click on the "Report" tab. Print/save the "Report" tab to a PDF file
 - 5.2.3 Upload all positive control PDFs to the "Attachments" tab of the MiSeq test batch.