Mitochondrial DNA Guidelines for mitoMPS casework		
Status: Published		Document ID: 51187
DATE EFFECTIVE 09/07/2021	APPROVED BY mtDNA Technical Leader	PAGE 1 OF 3

Mitochondrial DNA Guidelines for mitoMPS Casework

1 Purpose:

1.1 Guidelines for mitochondrial DNA testing to ensure clean laboratory practices, unambiguous sample identification, and relevant control runs.

2 General Procedures:

2.1 To reduce the possible contamination in the laboratory that could occur: (i) between the analyst and the samples (ii) from one sample to another, or (iii) from extraneous sources of DNA within the laboratory.

2.2 Lab coat, gloves, and mask, eye protection, and/or face shield, must always be worn while in the exam and pre-amplification room. Lab coat, gloves, eye protection must be worn in the post amplification area. All gowning must be done in the vestibules of exam, pre-amp or post-amp rooms.

2.3 Lab coats can be reused for a period of one week. Afterwards, they should be thrown out. Masks/face shields can also be reused for a period of one week. Goggles can be exposed to UV light in the Stratalinker to extend their time of use.

2.4 When working in the exam or pre-amplification laboratory, gloves must be rinsed in 10% bleach before each procedure and in-between the handling of separate samples.

2.5 Pipettes must be wiped down with 10% bleach before and after each procedure.

2.6 All hoods must be wiped down with 10% bleach before and after each procedure, followed by a 70% Ethyl Alcohol rinse, and UV light, if available, should be applied for 30 minutes before and following each procedure.

2.7 All racks, tube-openers and any other plastic implements (but not the pipettes) must be exposed to UV light in the Stratalinker for a minimum of 30 minutes before they can be used for amplification or extraction.

2.8 Any 96-well tube racks taken from the pre-amp room to the post-amp room must be placed into the post-amp bleach bath, rinsed, and dried prior to being returned to the pre-amp room.

2.9 The tubes used for washing, extraction and amplification must be exposed to UV light in the Stratalinker for 30 minutes. All 1.5 mL and 0.2 mL tubes will be closed and may be kept in plastic Nalgene boxes or comparable containers should only be removed with bleached and dried gloves while fully gowned.

2.10 Counters, sinks, refrigerator/freezer handles and door handles inside the laboratory and gowning room should be wiped down with 10% bleach on a monthly basis.

3 The mitoMPS workflow overview

3.1 Samples collected from evidence examination are processed through DNA extraction as warranted.

3.2 The mitochondrial control regions (hypervariable regions HVI, HVII and HVIII) are amplified into libraries using the Promega PowerSeq® CRM Nested Kit, in a process called library amplification.

3.3 The libraries are then purified, either manually or robotically, using Ampure XP magnetic purification beads, a process referred to as purification.

3.4 The purified libraries are quantified on a nM scale using the Promega PowerSeq® Quant MS kit, in a process referred to as quantitation.

3.5 Purified libraries whose quantitation value exceeds 4nM are diluted down to a 4nM concentration, and then quantified to confirm. A pool is created from aliquots of all libraries, in a process referred to as pooling.

3.6 An aliquot of this pool is then loaded onto a MiSeq FGx instrument, and run on a MiSeq FGx or MiSeq v300 60-cycle flow cell.

3.6.1 The run performs a clonal “bridge amplification” procedure on the surface of the flow cell and then sequences the libraries via fluorescent detection by the hardware.

3.6.2 The MiSeq FGx outputs the sequence information as FastQ datafiles, which are then imported into the Qiagen CLC Genomics workbench software with AFDIL-Qiagen Mitochondrial Expert (AQME) toolkit for analysis.

3.6.2.1 The sequences are trimmed for quality, aligned and then analyzed. Index tags introduced on the flanking ends of each library fragment identify the template of origin that produced that sequence.

3.6.2.2 Variant tables are then produced for each template whose libraries were sequenced.

4 Batching and Replication Guidelines

4.1 There is no restriction on the replication of samples. Performing multiple library amplification replicates of samples to bring the total number of libraries within a pool to 32 may be advantageous.

4.2 Evidence samples vs. Exemplar samples for processing.

4.2.1 Evidence samples from should be processed separately in time and/or location from exemplar samples through examination/sampling, DNA extraction, library amplification and purification.

4.2.2 Batching of evidence with exemplar samples is allowed during the library quantitation step, normalization step and quantitation of normalized libraries. Evidence and exemplar libraries may be combined into a single pool for loading and processing on the MiSeq, provided that the primer combinations used for the library amplifications of all samples are non-overlapping and compatible.

5 Nomenclature

5.1 The following are suggested naming conventions for use throughout the sample processing. The goal of this nomenclature is to ensure that sample names are unique identifiers.

5.1.1 Samples re-extracted for the purposes of replication (new cutting): The suffix: ”rep” will be added to the sample name to separately identify the re-extraction sample from the original, and this suffix will be applied to these replicate samples throughout the processing.

5.1.2 Samples reamplified in order to improve on the quality of the results or for other purposes: The suffix “reamp” will be added to the sample name. If multiple reamplifications are necessary, the numeral 2, 3, 4, etc. will be added to the suffix.

5.1.3 At the MiSeq run step:

5.1.3.1 The suffix “rerun” will be added to each pool that is reloaded onto the instrument for sequencing.

5.1.3.2 The index primers used will be added in alphabetical order as suffixes to each sample name. This suffix will always be added last, e.g. sample_D501_D701.

6 Repeat Analysis of Controls

6.1 If samples undergo repeat testing, consult below as to requirements for retesting of associated positive and negative controls.

6.1.1 Library amplification stage: New amplification negative and positive control must be included. The extraction negative control does not need to be repeated if it previously passed.

6.1.2 MiSeq: The original extraction negative does not have to be repeated if it passed for all needed sequences. The original amplification negative does not have to be repeated if it passed for all needed sequences. The original amplification positive control for the samples should be used in the pool, for each primer used; however, any positive control can also be used if the original is unavailable.