

# PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

Mitochondrial DNA Guidelines for mitoMPS casework		
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## Mitochondrial DNA Guidelines for MPS 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, 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.
- 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, followed by 70% ethanol 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% ethanol rinse, and a UV light, if available, should be applied for 30 minutes before and following each procedure.
- 2.7 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.
- 2.8 The tubes used for washing, extraction and amplification must be exposed to UV light in the Stratalinker/Crosslinker for 30 minutes. All 1.5 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 gowning.
- 2.9 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.

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## 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 MPS libraries are quantified on a pM scale using the Promega PowerSeq® Quant MS kit, in a process referred to as quantitation. Library concentrations are valid for a period of 7 days. If libraries have not been sequenced within this 7-day period, the libraries should be requantified prior to normalization and/or sequencing.
- 3.5 Purified libraries whose quantitation value exceeds 4nM are diluted down to a 4nM concentration and may be 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 v3 600-cycle flow cell.
- 3.6.1 A clonal “bridge amplification” procedure is performed on the surface of the flow cell and then the libraries are sequenced via fluorescent detection by the hardware.
- 3.6.1.1 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.
- 3.6.1.2 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.
- 3.6.1.3 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

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- 4.2.1 Evidence samples should be processed separately in time and/or location from exemplar samples through examination/sampling, DNA extraction, and library amplification.
- 4.2.2 Batching of evidence with exemplar samples is allowed during the library purification step, 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: "recut" should 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" should 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 The index primers used will be indicated with the addition of the sample well as a suffix to each sample name. This suffix will always be added last, e.g., sample name \_A1, sample name \_B1...
- 5.1.4 Index combinations will be rotated to achieve equal use of D7 primers. For each run, one set of primer combinations will be used (see Table 1 below). The following run will use the next set of primer combinations.

Table 1: Suffixes for Index Primers Combinations

PRIMERS	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D501	_A1	_A2	_A3	_A4	_A5	_A6	_A7	_A8	_A9	_A10	_A11	_A12
D502	_B1	_B2	_B3	_B4	_B5	_B6	_B7	_B8	_B9	_B10	_B11	_B12
D503	_C1	_C2	_C3	_C4	_C5	_C6	_C7	_C8	_C9	_C10	_C11	_C12
D504	_D1	_D2	_D3	_D4	_D5	_D6	_D7	_D8	_D9	_D10	_D11	_D12
D505	_E1	_E2	_E3	_E4	_E5	_E6	_E7	_E8	_E9	_E10	_E11	_E12
D506	_F1	_F2	_F3	_F4	_F5	_F6	_F7	_F8	_F9	_F10	_F11	_F12
D507	_G1	_G2	_G3	_G4	_G5	_G6	_G7	_G8	_G9	_G10	_G11	_G12
D508	_H1	_H2	_H3	_H4	_H5	_H6	_H7	_H8	_H9	_H10	_H11	_H12
	Set 1				Set 2				Set 3			

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5.1.5 At the MiSeq run step:

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

5.1.6 Following a MiSeq sequencing run:

5.1.6.1 Spaces, underscores, parentheses, and other special characters may be modified during the MiSeq run. Sample names prior to a MiSeq run may differ slightly from those generated following a MiSeq run.

## 6 Repeat Analysis of Samples and Controls

6.1 MPS libraries may be resequenced when unsatisfactory results are obtained. If resequencing >7 days from the most recent quantification, libraries (purified or normalized) should be requantified. Resequencing of the purified libraries is preferred if sufficient volume of purified library is available. If an insufficient volume of purified library is available, previously normalized libraries may be substituted.

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

6.2.1 Library amplification: New amplification negative and positive control must be included. Extraction negative controls should be reamplified.

6.2.2 Sequencing: All associated controls should be resequenced.

## 7 Sample and Data Storage

7.1 MPS libraries should be stored at -20°C.

7.2 MPS libraries should be stored for 6 months following the date of amplification. Libraries greater than 6 months past amplification may be discarded.

7.3 At minimum, FastQ files and SAV files from each sequencing run should be stored on the PowerWulf drive following run completion.

7.4 Fast-Q files from each run may be stored on the OCME network drive during analysis.