Mitochondrial DNA Guidelines		
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Mitochondrial DNA Guidelines

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 Nomenclature

3.1 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.

3.1.1 Samples re-extracted for the purposes of duplication (new cutting): The suffix: ”dup” 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 duplication samples throughout the processing.

3.1.2 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.

3.1.3 At the 3130xl run step:

- The suffix “recyc” will be added to each sample name for samples that are re-sequenced (e.g. sample-recyc). If multiple re-cycle sequences are necessary, the numeral 2, 3, 4, etc. will be added to the suffix.
- The suffix “conf” will be added to each sample name for samples that are re-sequenced to confirm sequence or length heteroplasmy (e.g. sample-conf). If multiple confirmatory sequences are necessary, the numeral 2, 3, 4, etc. will be added to the suffix.
- The primer used will be added as suffix to each sample name. This suffix will always be added last, e.g. sample_B4, sample_recyc_B4, sample_recyc2_B4, sample_conf2_B4.

3.1.4 Contig name:

3.1.4.1 A contig name should be the same as the LIMS sample name, followed by HVI/HVII identifiers, such as:

- FB17-01234_PM_1_1.1_bone-HVI
- FB17-01234_PM_1_1.1_bone-HVII
- FB17-01234_PM_1_1.1_bone_dup-HVI
- FB17-01234_PM_1_1.1_bone_dup-HVII

3.1.5 Sequencher ID

- The sequencher ID of an analyzed run will be identical to that run ID, e.g. S17001, B17-046.
- The sequencher ID of a case will be identical to the FB case number, e.g. FB1712345.

4 Repeat Analysis of Samples

4.1 Repeat testing of a sample can start at different stages, as listed below. Appropriate controls must be used.

4.1.1 Extraction stage: A new extraction negative control must be run.

4.1.2 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.

4.1.3 Cycle sequencing: Positive and negative controls must be tested for each primer used. 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; a cycle sequencing negative (cAN) should then be used (20 uL H_2O) for each primer used. The original positive control should be used and suffixed recyc for each primer used; however, any positive control can also be used as long as the contig of interest can be built with that positive control. Note that if a sample needs to be re-sequenced with a primer because the positive control at that primer failed, then every control or sample in that run needs to be re-sequenced with that primer.

5 Batching and Duplication Guidelines

5.1 Duplication of samples is only necessary from when samples are batched.

5.2 Exemplar samples batched and extracted for nuclear DNA may be duplicated with a second nuclear DNA extraction and STR typing.

5.3 For mtDNA, duplication of a given sample can be accomplished by running one informative primer for that sample in either HVI or HVII.

5.3.1 Evidence samples

5.3.1.1 Batching of evidence samples will be allowed at all steps of mtDNA analysis including the DNA extraction stage. Duplication at the extraction level can be done for case-related reasons (see supervisor).

5.3.1.2 Identical mtDNA profiles involving at least one evidence sample (two evidence samples or one evidence sample plus an exemplar) within a case are considered duplicated for the evidence sample.

5.3.1.3 Duplication of sample at the quantification level is not required. Evidence samples may be duplicated if they do not match any other sample in the case at the discretion of a supervisor.

5.3.1.4 Duplication of evidence samples may begin at the amplification or cycle sequencing steps if there is no additional evidence material for extraction or amplification, respectively.

5.3.2 Exemplar samples

5.3.2.1 Batching of exemplar samples from different cases will be allowed at all steps of mtDNA analysis including the DNA extraction stage.

5.3.2.2 HVI-HVII amplification and sequencing of exemplar samples from the same case (e.g. family members, duplication samples) should be performed at least once separately. Separate HVI-HVII amplification and sequencing of exemplar samples from the same case are not required if the mtDNA results are concordant with autosomal typing results, agreeing with an inclusion or exclusion.

5.3.2.3 Suspect exemplars may be duplicated at the discretion of a supervisor if that sample matches an evidence sample. Duplication of suspect exemplars is not required if the mtDNA results are concordant with autosomal results, agreeing with an inclusion or exclusion.

5.3.2.4 Victim exemplars may be duplicated at the discretion of a supervisor if they do not match any other sample in the case and if that exclusion is informative. Duplication of victim exemplars is not required if the mtDNA results are concordant with autosomal typing results, agreeing with an inclusion or exclusion.

5.3.2.5 Missing Persons' exemplars and unidentified remains do not need to be duplicated.

5.3.2.6 Any exemplar may be duplicated for case related reasons or to streamline testing.

5.3.3 Exemplar with Evidence samples

5.3.3.1 With the exception of quantitation, evidence and exemplar samples must always be tested separately in time and/or space. Batching of evidence

with exemplar samples is allowed during the DNA quantitation step. Batching of evidence with exemplar samples is also allowed during Agilent analysis provided that sample aliquots are done on each sample type (evidence or exemplar) at separate times. Quantification steps do not need to be duplicated.