

PROTOCOLS FOR FORENSIC MITOCHONDRIAL DNA ANALYSIS

EDITING GUIDELINES		
DATE EFFECTIVE 06-20-2016	APPROVED BY MITOCHONDRIAL DNA TECHNICAL LEADER	PAGE 1 OF 1

Editing Guidelines

Sequencher base calls can be modified if the underlying data support it. The analyst can change an “N” call into a base determination, insert an additional base, remove a position, or trim a sequence. A base call must not be edited without proper justification.

Reasons for base removal are:

- Extra base inserted due to broad peak, peak artifact, or analysis default spacing

Reasons for base insertion are:

- Base omitted however authentic peak is present
- To maintain proper spacing

Reasons for changing a base to an “N” or to a degenerate IUPAC code are:

- Ambiguous bases are detected
- Dye or electrophoretic artifact interference
- Due to sequence or length heteroplasmy

Reasons for changing an “N” call to a base is:

- Base omitted or called “N”, however authentic peak is present
- Dye artifact or electrophoretic interference
- Neighboring peak interference

Reasons for trimming a sequence:

- Trimmed to remove end sequence (sequence tail removal)
- Trimmed rCRS and sequences to other (shorter) sequence position for duplication

Editing for other reasons should be documented with a comment explaining the edit.

Many software calls can be easily resolved and corrected by the analyst. However, ambiguous situations should not be edited. If an electrophoresis problem is suspected, this sample should be re-injected. Sequence information at each base position should be confirmed by data from both DNA strands when possible. Single-stranded regions present due to length heteroplasmy, must be confirmed by confirmatory sequencing of the same strand in the same direction. The Sequencher complementary strand alignment will flag conflicts between the two sequencing directions for all strands imported into the contig.