Analysis Using Sequencer

Launch Sequencher 4.1.4Fb19.

Import the sequences analyzed in Sequencing Analysis: File→Import→Sequences.

Open each sequence individually by double clicking on it. Edit each sequence by trimming off the dirty ends. Do so by highlighting the top row of bases and clicking delete. Make note of any sequences that are not analyzable.

For those sequences that are not analyzable, refrigerate them by selecting Edit→Refrigerate→Name. The name should include the sample designation, case #, item #, and NA for not analyzable. Proceed to analyze those sequences that are analyzable.

Highlight each analyzable NC and hit 'Assemble Automatically'. A contig will be produced. For each contig produced, name it with the corresponding sample designation, case #, and item#. Open each contig individually and look at primer direction. A's/C's should be going in the forward (green) direction, and B's/D's should be going in the reverse (red) direction. (Another option is to try assembling with the rCRS, which is helpful for samples with an HV1 C-stretch).

Click on the box labeled 'Bases' to observe the nucleotide sequence. Highlight a base and click on 'Show Chromatograms' to observe the electropherograms. Delete the primer sequences: HV1 begins with AAGATTC and ends with ATAGGG; HV2 begins with ATTTGGT and ends with ATCTTT; HV1A begins with AAGATTC and ends with TATCACA; HV1B begins with ATAAAAA and ends with ATAGGG; HV2A begins with ATTTGGT and ends with ATCATAA; HV2B begins with ATTACAG and ends with ATCTTT.

*For samples where an HV1 C-stretch occurs: use the forward strand and count 14 bases beginning with the group of 4 A's starting at base position 16180. Cut off the rest of the sequence. Make note of the number of C's in the forward strand (typically 4 A's followed by 10 C's if a base change at 16189 occurs). For the reverse strand, beginning at base position 16193 use the number of C's in the forward strand and count back. Cut off the rest of the sequence. Typically the truncated range within this area is 16180-16193 ($A_4C_5TC_4$), barring any insertions or deletions.

Each base, in both strands (if applicable), must be manually reviewed. Unambiguous bases must be labeled using the IUPAC codes.

Once editing is completed, import the rCRS into the project. Highlight the rCRS and the relevant contig and click on 'Assemble Automatically'. A new contig will be created which will consist of the sample's consensus sequence and the rCRS.

View the differences from the rCRS using the 'Difference Review': Contig→Difference Review. Export the Difference Review as a text, saving it to a designated folder.

Open the saved 'Difference Review' and add the Case #, Item #, Examiner's initials, Date, and sequence Ranges. Print the file and re-save it.

Repeat for RB's, Samples, and Positives.

For any sequence that is not being used (i.e. poor quality, mixtures), refrigerate it and name it with the case#, item#, and DNU for did not use.

Save the Sequencher project to include case# and item#.

Expand the view of the Sequencher project: View→Expand All.

Go to File→Set Header and Footer→change Top to say 0.2in. Go to Print Setup→Change Orientation to Landscape. Go to Properties→Finishing→2 pages per sheet. Print the Sequencher project.

The analysis packet should include: Difference Files for the Sample, Positive Control, and any RB's/NC's which went into a contig, and the Sequencher Project.

Note: Mixture samples will be noted within the case workbook. A notation about the performance of the controls will be made. An analysis packet will NOT be printed out for mixture samples, nor will second analysis by another qualified mtDNA analyst occur.

*Refer to mtDNA SOP 09 for further instructions on sequence nomenclature, comparisons, length heteroplasmy, and sequence ranges.