Validation and Application of New Sequencing Primers for Use in Mitochondrial DNA Control Region Analysis

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The objective of this presentation is to introduce alternative strategies for sequencing the Control Region of the mitochondrial genome. Initial efforts at sequencing the entire mitochondrial Control Region quickly demonstrated the need for alternative sequencing primers targeting the VR2 region. Initially, two primers (F155 and R599) were used to sequence the VR2 region. In samples where the problem of length hetroplasmy within the HV2 C-stretch was encountered, sequencing data generated by the F155 primer could not be used. In these cases, R599 is the only source for sequencing data in the VR2 region. At the Armed Forces DNA Identification Laboratory (AFDIL), data is confirmed when both a forward and reverse primer or two different amplifications or extractions using the same sequencing primer provide quality data with no unconfirmed positions, as explained in AFDIL's protocol for sequencing analysis. The quality of data provided by mtDNA sequencing using R599 has generally been of lesser quality, when compared to mtDNA sequencing of non-c-stretch templates using F155. Due to the lack of forward primer sequencing data for this region when HV2 C-stretch problem is present, a validation study was conducted to determine sequencing quality of three new VR2 sequencing primers (F311, F314, and F320) that bind within the HV2 C-stretch region and are unaffected by length heteroplasmy in this region.

This study involved applying F311, F314, and F320 to ten samples where sequencing of the entire Control Region was employed. Four samples involved sequencing data where the HV2 C-stretch problem was encountered, while another four samples did not show evidence of this problem; the remaining two samples involved poor quality extracts. Using F311, 80% of the samples were successfully sequenced, using F320, 90% of the samples were successfully sequenced, and when using F314, 100% of the samples were successfully sequenced. Eight of the ten samples were successfully sequenced by all three primers. When analyzing these eight samples, F311 generated 96.2% of the total possible sequence, F320 generated 98.3% of the total possible sequence and F314 demonstrated the greatest efficiency by generating 99.0% of the total possible sequence. Of all three primers, F314 seemed to be the superior primer, even though all of them sequenced extremely well through the VR2 region and overcame the HV2 C-stretch problem. All three primers should be taken into consideration for use on a case by case basis.

Due to the fact that F314 demonstrated a marked improvement over the existing primers in a study of cases involving varied sequence quality and polymorphisms, this primer is being applied to numerous samples within a Control Region database to confirm VR2 data. The Control Region database includes samples that exhibit HV2 C-stretch problems as well as lesser quality R599 sequencing data. In addition, other primers are being investigated to confirm other areas within the control region. Data from this study is currently being compiled and will be presented.

In conclusion, HV2 C-stretch length heteroplasmy, encountered when sequencing the mtDNA Control Region is not uncommon. In these cases, the sequencing quality of the VR2 region is diminished. With the development of three new VR2 sequencing primers (F311, F314, and F320), these problems can be overcome to produce above average results.