Evaluation of the diversity whole mtDNA in a sample from Brasilia-DF, Brazil

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Introduction. Most forensic laboratories that use mtDNA typing are based on the polymorphisms present in the nucleotide sequence in the hypervariable region, comparing the unknowed sample with the reference sequence (rCRS) for the annotation of the polymorphisms.

However, it has been reported that about 75% of the total mtDNA variation occurs outside the control region and the whole mtDNA sequencing would increase the discrimination power and the value of the generated data. In a study with Hispanic, Caucasian and African-American individuals, the genetic diversity for these three groups of individuals was averaged 98% when only the HV1 and HV2 regions were analyzed and on average 99.9% when the whole mtDNA was analyzed. The genetic diversity in Caucasian individuals was 100% in the analysis with the whole mtDNA [1].

In a population sample from Estonia, the genetic diversity obtained with only analysis of the HV1 and 2 regions was 95.85%, while the analysis with the whole mtDNA increased the diversity to 99.67% [2].

This increase in the genetic diversity obtained when analyzing the whole mtDNA has been observed in different world populations [3] and can make more efficient the classification of haplogroups according to ethnic origin (PARK, 2017).

Objectives. Evaluate if the analysis of the whole mitochondrial genome by massively parallel sequencing (MPS) can increase the diversity in samples that presented shared haplotypes in the analysis of the mtDNA control region by capillary electrophoresis.

Material and methods. We selected 22 blood samples from natural individuals from Brasilia-Federal District (Central-West region of Brazil) that had the entire mtDNA control region analyzed by Sanger sequencing using M13-tailed primers L15997+M13F and H639+M13R and presented 8 shared haplotypes. The whole mitochondrial genome was analyzed by MPS: amplification of the mtDNA by long PCR in two separate reactions using the TaKaRa LA PCR Kit (TaKaRa) and library preparation using the Nextera XT DNA Sample Kit (Illumina).

Results and discussion. The population of the Central-West region is highly mixed due to its historical formation and nowadays has a scarcity of mtDNA data. Analyzing only the hypervariable region, 8 haplotypes were identified in a total of 22 samples. The whole mtDNA analysis allowed the identification of 18 haplotypes, 4 of which were shared only by two samples (Fig. 1).

These are preliminary data, as part of a research that seeks to evaluate the whole mitochondrial genome in samples of Brazilian admixture population, but nevertheless, it is possible to identify that the analysis of the whole mtDNA can increase the diversity of haplotypes found in our population samples.
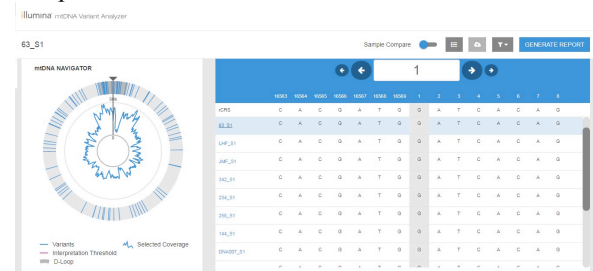

Figure 1: *Example of the whole genome analysis in the mtDNA Variant Analyzer software (Illumina). To the left side is shown the depth of reading obtained in each region of the genome and to the right side the sequence obtained..*

Conclusions. As expected, the methodology used was efficient for analysis of the control region and the whole mtDNA and, all data obtained for the control region by Sanger sequencing were confirmed by MPS. The complete mtDNA analysis significantly increased the diversity for the samples in study.

References.

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