MITOCHONDRIAL CONTROL REGION ANALYSIS BY MASSIVELY PARALLEL SEQUENCING USING POWERSEQ™ MITO CONTROL NESTED SYSTEM

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Massively parallel sequencing allows laboratories access to mitochondrial DNA (mtDNA) analysis using a more sensitive and high-throughput workflow compared to traditional sequencing methods. Increased mixture deconvolution and heteroplasmy resolution are achieved by deep sequencing coverage and digital read counts. Additionally, the use of small amplicons to sequence the mitochondrial control region improves sequencing results from degraded samples. However, library preparation for many massively parallel sequencing workflows require multiple enzymatic and purification steps that are time consuming and often a source of variability and sample loss. The prototype PowerSeq™ Mito Control Nested System utilizes a nested amplification protocol that greatly reduces the number of steps and time required to produce libraries ready for sequencing. The protocol consists of a single PCR step to both amplify the target amplicons and incorporate indexed sequencing adapters. The system generates 10 small amplicons (adapted from Eichmann and Parson) covering the control region of the mitochondrial genome in one multiplex. The targeted regions for amplification are designed to be in a range of 140-250bp to ensure optimal results from degraded samples. We will demonstrate this improved workflow for the nested amplification of mitochondrial HVI and HVII control regions.

1. Eichmann C., Parson W.: “Mitominis”: multiplex PCR analysis of reduced size amplicons for compound sequence analysis of the entire mtDNA control region in highly degraded samples, *Int J Legal Med*. 122 (2008) 385-8.