Massively parallel sequencing (MPS) is a tool with many advantages for mitochondrial DNA (mtDNA) analysis, including increased throughput from running multiple samples simultaneously and greater resolution for detecting mixtures and heteroplasmy when compared to more traditional sequencing methods. The PowerSeq™ Whole Mito System is designed to enable analysis of the entire human mitochondrial genome using MPS. It includes reagents for amplification of the entire 16,569 bp mitochondrial DNA sequence in a single multiplex reaction, generating 161 small amplicons. The use of small amplicons (167 bp average) allows for more robust analysis of degraded samples where nuclear DNA may be of insufficient quantity or quality. Here we demonstrate that the PowerSeq™ Whole Mito System is both accurate and sensitive, giving full mitochondrial DNA profiles with as little as 60 pg of input DNA.