Certain types of evidence require mitochondrial DNA (mtDNA) sequencing to obtain DNA results suitable for comparison. Sanger sequencing methods are time consuming and may require additional equipment, instrumentation or space. Traditional library preparation protocols for massively parallel sequencing (MPS) workflows are time consuming and require multiple enzymatic and purification steps which could introduce variability and sample loss.

The PowerSeq™ CRM Nested System simplifies the massively parallel sequencing approach for analysis of the human mtDNA control region. Multiplex amplification produces 10 small amplicons (adapted from Eichmann and Parson) covering the control region. These amplicons range in size from 144bp to 237bp to ensure optimal results from degraded samples. The targeted regions are amplified while indexed sequencing adapters are incorporated during a single PCR reaction. This nested protocol greatly reduces the library preparation steps and time needed to produce libraries ready for sequencing.

MPS analysis of the human mitochondrial control region provides many benefits and opportunities for the forensic community: analysis of challenging samples; high-throughput sample processing; and digital read counts assist analysis of mixed DNA samples and heteroplasmy. The PowerSeq™ CRM Nested System provides the many advantages of MPS with the added benefits of its streamlined workflow.