OPTIMIZATION, AN OLD QUESTION REVISITED

Timothy P. McMahon Ph.D.
Armed Forces DNA Identification Laboratory, Rockville, MD

A common strategy in the forensic field for the identification of ancient skeletal remains is the isolation and sequencing of mitochondrial DNA (mtDNA) hypervariable regions 1 and 2 (HV1 &HV2). The high copy number of mtDNA genomes contained within the cytoplasm of cells allows for identification of individuals where little to no nuclear DNA would be isolated. This strategy helps the mitochondrial section of the Armed Forces DNA Identification Laboratory (AFDIL) accomplish its primary mission to aid the Central Identification Laboratory in Hawaii (CILHI) with the identification of human skeletal remains recovered from World War II, the Korean War, and the Vietnam conflict. Currently we amplify HV1 (nt15989-16410) and HV2 (nt15-389) regions with GeneAmp 10X PCR buffer (Applied Biosystems), AmpliTaq Gold polymerase (Applied Biosystems) and either four primer sets for relatively intact mtDNA genomes or 8 mini-primer sets for highly degraded or inhibited samples. In experiments described here extracts representing a range of sample qualities were used to optimize and validate GeneAmp 10X PCR Gold buffer (Applied Biosystems) for use in casework. Initially, 1.5 ul of AmpliTaq Gold polymerase and GeneAmp PCR Gold buffer containing 1-4mM $MgCl_2$ was used to identify the optimal $MgCl_2$ concentration for each primer set. Results suggested that there was little difference between amplicon yield with either GeneAmp PCR Gold buffer at 1-4 mM $MgCl_2$ or GeneAmp PCR buffer when positive control DNA (200pg) was used. However, GeneAmp PCR Gold buffer with final concentrations of $MgCl_2$ at 4mM for PS1 (nt15989-16251) and 3mM $MgCl_2$ for PS2 (nt16190-16410), PS3 (nt15-285), and PS4 (nt155-389) gave slightly increased amplicon yield with uninhibited case extract. Next, using optimized $MgCl_2$ conditions we observed that when 2.5 ul of AmpliTaq Gold polymerase was used the amplicon yield with GeneAmp PCR and GeneAmp PCR Gold buffers were similar, thus suggesting that we could use less AmpliTaq Gold polymerase in our optimized amplification reactions to obtain the same results. Finally, we demonstrated that these optimized amplification conditions allowed us to obtain amplicons with primer sets from inhibited case samples previously amplified with mini-primer sets. Taken together, these results demonstrate the need to re-evaluate optimal conditions for established protocols when new and improved reagents become available. The continued identification of optimal reaction conditions allows us to more efficiently accomplish our primary mission of identifying human remains, by increasing productivity and decreasing turn around time of case samples.