OPTIMIZATION OF PROTEINASE K, INCUBATION TIME, AND AGITATION IN GENOMIC DNA ISOLATION FOR HIGH THROUGHPUT EXTRACTIONS

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Genetic testing laboratories must maintain standard protocols for DNA extraction, and balance time and cost versus yield when optimizing protocols. We explored the relationship between proteinase K concentrations, incubation times, and agitation during incubation, as well as their cumulative affect during tissue lysis on DNA yield. These factors may complement each other, allowing for more complete tissue digestion, and thus, higher yield in DNA extraction, or they may exhibit redundant properties. Our laboratory uses a modified extraction protocol with Qiagen’s Biosprint 96 DNA Blood Kit on human buccal swabs. Here, we assessed the roles of these three factors of interest, and how they work together. We performed this work using a homogenous solution of blended beef liver in lysis buffer to provide a large volume with a consistent sample concentration for all trials. To evaluate each trial’s DNA yield, all samples were quantified using a FLX800 Florescence Microplate Reader. We visualized DNA using agarose gel electrophoresis to assess its quality. To test for co-purification of PCR inhibitors, we amplified the DNA isolates of the human buccal swabs on an STR multiplex. The results of this work provide insight for the design of DNA extraction protocols to balance time and cost with sample yield.