

EVALUATION OF A NOVEL DNA EXTRACTION TECHNIQUE, FOR OPTIMAL RECOVERY OF DNA FROM EPITHELIAL CELLS

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In the field of forensic science, there is a constant demand for new and improved techniques to enhance the information one can gather from the biological trace evidence. Humans shed approximately 400,000 epithelial cells daily which can be a useful source of DNA evidence. However, DNA obtained from touch samples, that leave only a few cells behind can prove to be difficult as the collection and the extraction process result in significant loss of valuable DNA. It is therefore essential that the procedures from sample collection to DNA extraction to PCR and genetic profiling be optimized to ensure the best possible results from low abundance samples. This study is a comparative analysis between commercially available Qiagen DNA extraction kits and the novel extraction technique, pressure cycling. Pressure Cycling Technology (PCT) uses rapid cycles of hydrostatic pressure ranging from ambient to ultra-high levels to disrupt biomolecular interactions. Cultured, keratinocytes were re-suspended in basal growth media, counted using a hemocytometer and approximately 10,000 cells were placed into tubes as well as spotted directly onto 1.2µm polycarbonate filters and the Qiagen MicroKit DNA extraction protocol was performed. For comparison 10,000 cells was placed directly into the pressure cycling tubes as well as onto filters, the filters then placed into pressure cycling tubes and 1.5mL buffer ATL (Qiagen) and 20µL of Proteinase K (>600 mAU/mL) were added. These samples were subjected to 20 cycles of 0 to 35000 PSI using the Barocycler NEP2320. Following the initial PCT step, samples were subjected to the Qiagen column clean up per manufacturers' protocols. The two methods were compared using quantitative PCR and the addition of pressure cycling to the standard Qiagen protocols resulted in an increase in DNA yield of 16%, when pressure cycling is used vs Qiagen alone. Further experiments with dilution series of cells ranging from 10,000 to 50 cells as well as touch samples collected on porous and non-porous surfaces is currently being tested.