B57 The Optimization of Human Hair Proteomic Processing for Single Hair and Ancestral Analysis

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After attending this presentation, attendees will understand the impact of using genetic information from hair peptides to aid in human identification. Attendees will also realize why proteomic processing is important in generating meaningful proteomic datasets.

This presentation will impact the forensic science community by shifting human identification into a dual DNA-proteomics perspective. Further implications will also be made in making identifications and ancestral classifications based on genetic information found in protein from a single human hair.

Forensic hair evidence obtained by microscopic morphological comparison has been criticized as being subjective, unreliable, and not reproducible. Mitochondrial DNA analysis has been able to remedy the reliability of hair analysis, but DNA can degrade substantially under environmental insults, particularly with greater distance from the scalp. Unlike DNA, protein has peptide bonds that are more resistant to cleavage. This research explores a proteomic approach to hair analysis. Variations in protein expression level reflect variations in transcription factor levels, their affinities for response elements, and possibly epigenetic and genetic variation. Another form of variation is the presence of Genetically Variant Peptides (GVPs) that offer the prospect of even more discriminating analysis.[1]

GVPs are the result of non-synonymous Single Nucleotide Polymorphisms (SNPs); therefore, genetic information such as SNPs can be predicted via the protein sequence and confirmed with parallel DNA sequencing. With high-resolution mass spectrometric instrumentation, single amino acid polymorphisms can be detected in the amino acid sequences of keratin, keratin-associated, and other proteins. As a result, GVPs can be used to help identify an individual or even classify ancestral origin.

This research focuses on increasing peptide yield from hair protein by optimizing its chemical processing. Further goals include comparing European and African hair GVPs and assigning the differences in population frequency found therein, as well as decreasing the working hair length to 20mm (~100µg). Data have been obtained to optimize conditions for disulfide reduction, alkylation, and peptide digestion. Temperature, time, agitation types, and concentrations of the reagents have been tested. The four metrics used to determine the best parameters for processing are yield of the insoluble fraction, yield of the soluble fraction, unique peptide number, and the number of GVPs. Results indicate that lower temperatures were better than higher temperatures. Agitation by stirring resulted in higher solubilization than swirling or remaining static. A time course quantifying the insoluble fraction has shown that trypsinization for six hours solubilizes most of the hair by mass and results in detection of the most unique and total peptides. The optimized hair processing procedure, with shorter times for both reduction and digestion, has yielded improvements in detectable GVPs, and yields similar numbers of GVPs compared to other approaches that rely on urea and a mass spectrometry compatible detergent; however, the increase in peptide and GVP generation is more pronounced in European hair as opposed to African hair. This is mainly due to the lower quantity of keratin-associated proteins detected in African hair. Overall, the data show optimized proteomic processing of human hair results from shorter reduction and digestion at room temperature with gentle stirring.

Reference(s):

1. Parker, Glendon J., Tami Leppert, Deon S. Anex, Jonathan K. Hilmer, Nori Matsunami, Lisa Baird, Jeffery Stevens et al. Demonstration of protein-based human identification using the hair shaft proteome. *PlOS One.* 11, no. 9 (2016): e0160653.

Proteomics, Hair, Genetically Variant Peptides

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