Optimizing Peptide Yields From Human Hair

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Abstract

Hair is physically robust and persists in the environment for long periods. It is a common component of crime scenes, yet it is not often analyzed because the DNA information is absent or difficult to obtain. Hair, a complex biological connection between the crime scene and the suspect, is now only rarely used in crime scene investigation. This project seeks to examine the use of protein in developing a measure of identity, we currently have a power of discrimination of 1 in 15,500. The degree of identity between an individual and a hair sample is a function of proteomic information obtained.

The purpose and question of our research is to determine if there is a more efficient way to optimize peptide yields from hair samples. This will maximize the amount of useable information as a measure of identity in forensic cases.

Background

DNA-based methodology has revolutionized forensic science. It has expanded the amount of information that can be extracted from forensic samples, and it is quantifiable, providing statistically based measures of identity with given probabilities and ranges of certainty [1, 2]. Unfortunately, DNA is a relatively weak molecule that easily degrades and is undetectable in many forensic contexts [3, 4]. This project proposes to extend the utility of DNA methodology through the analysis of protein variation. Since the primary structure of protein is a function of DNA, proteins retain evidence of DNA variation well after the DNA template is removed through physiological, environmental or chemical processes [5-7]. We propose to detect these peptide variants as genetic biomarkers for non-synonymous DNA single nucleotide polymorphisms (SNPs).

Methods

Hair is incubated in 10% methanol, 1 mM DTT, 50 mM NH₄HCO₃ at 95°C for 16 hours and then rinsed in 10% methanol. Hair was lyophilized in speed vac for 4 hours and then ground into fine powder. Hair powder was then incubated with trypsin under various conditions for 48 hours at 37°C. Trypsinized samples are centrifuged for 20 minutes at 15300 x g and supernatant was removed. Samples were applied to a C18 reverse phase column on a Agilent HPLC and eluted with ACN 0.1% TFA (mobile phase) gradient while measuring absorbance at 214 nm. Samples were also sent to University of Utah for mass spectrometry analysis.

Results

Many SNPs have known allelic frequencies, and, therefore, can provide an alternative method for quantifying identity [8]. Likewise, protein / peptide polymorphisms also have the potential to provide a random match probability. Because proteins are more stable, this methodology has the potential to be applied when DNA cannot be analyzed, such as in hair shafts, and environmentally compromised or aged samples.

Conclusions

The analysis needs to be conducted on a larger number of individuals, the identified informative SNPs need to be directly confirmed, mass spectrometry assignments should be confirmed using synthetic peptides. We also need to develop software to automate the analysis of mass spectrometry data and calculate probabilities of association and random match probabilities. Finally we need to broaden the methodology to include other tissues and individuals of biogeographic backgrounds that are not Northern European.

References


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Future Research

This project seeks to examine the use of protein in developing a measure of identity. The purpose is to Determine if there is a more efficient way to optimize peptide yields from problematic protein samples. This methodology will increase the amount of useable information as a measure of identity in forensic relevant material.