B171 Age Determination: The Identification of Newborns Using Messenger RNA Profiling Analysis

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Attendees will learn a method to determine if a bloodstain originated from a newborn baby.

This presentation will impact the forensic community and/or humanity by demonstrating the development of a novel method, which can be incorporated into the forensic laboratory, to aid in investigations concerning bloodstains thought to originate from newborns.

It is now a matter of routine for the forensic scientist to obtain the genetic profile of an individual from DNA recovered from a biological stain deposited at a crime scene. Potential contributors of the stain must either be known to investigators (i.e., a developed suspect) or the questioned profile must be searched against a database of DNA profiles such as those maintained in the CODIS National DNA database. However, in those instances where there is no developed suspect as yet or there is no match with any database sample, the DNA profile *per se* presently provides no meaningful information to investigators, with the notable exception of gender determination.

To aid in these investigations another useful biometric that could provide important probative information is the age of an individual. For example, the ability to provide investigators with information as to whether a DNA donor is a newborn baby, an adolescent teenager or an elderly individual could be useful in certain cases, particularly those involving young children such as kidnapping or in providing additional intelligence during terrorist investigations. Currently no reliable validated molecular tests are available for age determination.

The lifecycle of humans comprises a number of developmentally recognized stages. As the human proceeds through these developmental stages, sub-sets of the 30-50 thousand human genes will be differentially expressed. Theoretically, and given sufficient knowledge of developmental genetics, a determination of the global gene expression profile could reveal constellations of genes whose expression is correlated with a specific age.

One example of how developmental regulation of gene expression can lead to the determination of age is by examining the ß-hemoglobin locus located on the short arm of chromosome 11 (11p15.5). This chromosomal region encodes five functional ß-like globin genes, ε, γ^G, γ^A, δ and ß, each with a specific pattern of highly regulated developmental gene expression. In the first weeks of neonatal development embryonic hemoglobin (ξ^2, ε^2) is produced by the yolk sac. Around twelve weeks of gestation embryonic hemoglobin synthesis decreases and the fetal liver, spleen, and bone marrow begin producing fetal hemoglobin (α^2, γ^2), which continues throughout fetal development. Shortly after birth fetal hemoglobin production decreases and the synthesis of adult hemoglobin (α^2, $ß^2$) rises and is the major form of hemoglobin present throughout life. Therefore, the development of an assay which selectively identifies an increased presence of fetal hemoglobin (γ chain) in a biological stain, would infer that the donor of the stain is a newborn baby.

Descrived here are two novel methods which assay the age related levels of variant forms of gamma hemoglobin (HBGv) present in bloodstains, with an increased level of expression being indicative of the newborn status of the individual. The first method is a duplex reverse transcription-polymerase chain reaction (RT-PCR) and allows for the determination of a newborn based on a present / absent gamma hemoglobin product, along with the presence of an internal control, the ribosomal protein S15. A second realtime-PCR (qPCR) assay, can distinguish between newborns and other age groups by evaluating the ΔC_t values (C^t S15 - C_t HBG) generated. A positive ΔC_t value would indicate that a bloodstain originated from a newborn; in contrast a negative ΔC_t value would be obtained with all other ages.

Age Determination, Identification of Newborns, Messenger RNA Profiling

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