

Y-CHROMOSOME SPECIFIC NESTED PCR PRE-AMPLIFICATION METHOD FOR IMPROVED DETECTION OF MALE DNA

Erin Hanson Ph.D.¹, Mindy Solivan¹, M.S., Sascha Strauss², Ph.D., Francesca Di Pasquale², Ph.D., Holger Engel², Ph.D. and Jack Ballantyne^{1,3}, Ph.D

¹National Center for Forensic Science, PO Box 162367, Orlando, FL 32816-2366

² QIAGEN GmbH, QIAGEN Str.1, 40724 Hilden, Germany

³University of Central Florida, Department of Chemistry, PO Box 162366, Orlando, FL 32826-2366

Male DNA-containing samples collected from sexual assault or homicide victims can contain very low levels of cellular male DNA admixed with a large number of female epithelial cells. This often results in failure to obtain an autosomal STR typing from the male DNA donor. Y-STR analysis can be used to overcome this problem. However there are still many instances where such an approach does not work. This is particularly so when an intimate sample is collected many days after the incident, usually as a result of delayed reporting by a rape victim or when there is a significant time interval between death and recovery of a rape/homicide victim's body or when the samples manifest some degree of degradation. Recent technological advances in the area of DNA profiling offer the opportunity to improve the number of specimens that can be successfully analyzed. Therefore it may be possible to develop strategies to overcome the problems associated with low levels of male DNA in a background of female DNA. We have developed such a method using a selective amplification of Y chromosomal genomic DNA prior to standard Y-STR analysis. This 'genomic partitioning' appears to be an effective strategy to further increase the signal to noise ratio of the Y chromosomal DNA compared with the epithelial DNA and hence allow clear unambiguous male profiles to be obtained. Additionally, such an approach could also be used to improve the analysis of touch or contact DNA samples which often contain small amounts of male DNA.

In this work, we have developed a 17-locus Y chromosome specific nested PCR pre-amplification multiplex and have performed an initial validation to demonstrate its potential suitability for use with forensic samples. The pre-amplification takes less than 2 hours to perform and can be used in conjunction with commercially available Y-STR amplification kits. The use of the nested PCR pre-amplification prior to Y-STR analysis allows for the recovery of Y-STR profiles from as little as 5 pg of male DNA (~ 1 diploid cell) from various body fluids and tissue (blood, semen, saliva and skin). No interference from female DNA was observed even in the presence of female DNA in 100,000-fold excess. We have demonstrated the method's ability to recover Y-STR profiles from touch and contact DNA samples as well as extended interval post coital samples (≥ 5 days after intercourse).

Our poster will describe the development and validation of the chromosome specific nested PCR amplification method as well as performance with forensic casework-type samples such as touch/contact and extended interval post coital samples.