SEROLOGY BY qPCR ARRAY

Anissa Wiley and Mark F. Kavlick
Counterterrorism and Forensic Science Research Unit, Laboratory Division, Federal Bureau of Investigation, Quantico VA, USA

A qPCR array was designed to facilitate forensic serological identification of unknown stains and fluids. In our approach, the flow-through of a DNA purification column is further purified for total RNA. Therefore, the DNA preparation is fully available for forensic DNA analysis whereas the RNA fraction is processed for serological examination by mRNA analysis. The RNA fraction is first subjected to DNase treatment to remove contaminating DNA and then reverse-transcribed to generate cDNA which is subsequently assayed by real-time (qPCR). The cDNA preparation is simply added to a qPCR master mix and dispensed into a qPCR array containing 32 separate assays, each of which contains primers and probe for a specific gene transcript. The transcripts queried in the array represent candidate gene markers for semen, seminal fluid, saliva, blood, menstrual blood, vaginal secretions, skin, sweat, and urine. The specimen types evaluated included all of the above except for sweat. In addition, up to six assays for housekeeping genes were included in the array to normalize the quantity of RNA extracted. The qPCR arrays were pre-loaded in triplicate on a 96-well plate therefore up to three different cDNA preparations may be simultaneously tested per plate or a single cDNA preparation may be tested in triplicate. Sample loading is accomplished in minutes and qPCR run time is completed in approximately 50 minutes. Following qPCR, amplification plots, Ct values, and ΔΔCt values are analyzed.

A comparison of amplification plots revealed that most candidate gene markers were expressed as expected for their respective specimen types. However, some markers were also found to be expressed to some degree in unrelated specimen types, suggesting the need for alternative markers that are highly-specific for each specimen type. In particular, many of the candidate urine markers evaluated were also expressed in non-urine specimens indicating the need to evaluate alternative mRNA markers for urine. Furthermore, nucleic acid extracts of urine and skin specimens contained relatively low RNA levels and thus exhibited low gene expression, suggesting the need to identify alternative markers for those specimen types which are highly-expressed. Expression of housekeeping genes varied considerably among the housekeeping genes tested as well as among the different sample types tested. However, the 18S rRNA housekeeping gene was consistently among the most highly-expressed for all sample types, suggesting its suitability for samples containing low quantities of RNA. Finally, various calibrators or control samples for use in the ΔΔCt analysis of forensic-type specimens were evaluated, including cDNA preparations of non-forensic specimens such as normal human tissue and cell lines.

qPCR arrays as described here may be used to identify an unknown stain or fluid as being semen, seminal fluid, saliva, blood, menstrual blood, vaginal secretions, skin, or urine. This single test is rapid, uses instrumentation common to a forensic laboratory, and can potentially replace a variety of chemical, biochemical, and immunological tests for these specimen types. However, more efforts are needed to identify highly-expressed and highly-specific specimen markers for forensic serological analysis by qPCR array, particularly for low copy specimens such as skin cells and urine. ⌘