

PHYSICAL SEPARATION AND STR ANALYSIS OF FORENSIC MIXTURES USING LASER MICRODISSECTION AND FLUORESCENT IN SITU HYBRIDIZATION

Robert Driscoll M.F.S., Dane Plaza B.S., and Robert Bever Ph.D.

Bode Technology, Lorton, VA

Mixtures from two different individuals cannot be easily resolved when the mixtures consist of similar cell types. Laser microdissection (LM) has been successful in physically separating sperm cells from epithelial cells in order to resolve DNA mixtures associated with sexual assault evidence. Often assault evidence involves cells not associated with spermatocytes but contains mixtures of epithelial or blood cells. For the successful resolution of these mixtures, we have implemented a four step procedure to resolve mixtures of male and female epithelial cells or male and female blood cells. The four step protocol consists of the following methods:

1. Identification of male and female cells using fluorescent in situ hybridization (FISH) techniques with the Vysis CEP X[®] alpha satellite and CEP Y[®] satellite III probes.
2. The physical separation and collection of male and female cells using the Arcturus[®] PixCell II or Zeiss PALM[®] MicroBeam Systems.
3. DNA extraction using Qiagen QIAamp[®] DNA Micro collection kits.
4. The amplification of the LM collected cells using Applied Biosystem Inc. (ABI) AMFISTR Identifiler[®] and/or ABI AMPFISTR MiniFiler[®] systems.

Using the above protocol, STR profiles have been resolved from male/female mixtures of epithelial cells, white blood cells, and combinations of both. The utilization of low volume ABI Identifiler[®] and MiniFiler[®] amplification reactions has allowed for the generation of full and partial STR profiles originating from 500 to 5 FISH processed cells collected by laser microdissection. The entire process can be performed within 16 hours. Additionally we have used this procedure to develop single STR profiles from blood and saliva stains placed on paper substrates, cotton fabric, and stainless steel surfaces.