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SEPARATION OF DIPLOID CELLS USING FISH AND LMD APPLICATIONS IN THE ANALYSIS OF SEXUAL ASSAULT EVIDENCE

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Mixtures of DNA from different individuals are a common occurrence in many forensic cases and due to the mixtures often cases are not able to be resolved. Approximately 20% of cases worked in 2004 at the North Louisiana Crime Lab resulted in a mixture in the DNA profile from the crime scene evidence. These mixtures are often time-consuming and difficult to interpret. A profile from a piece of evidence yielding a mixture typically won't be accepted into the Combined DNA Index System (CODIS), the DNA national database. Sexual Assault cases, which are the most frequent type of case submitted to crime labs, often have evidence that contain a mixture of DNA from a female individual and a male individual. Currently there is no protocol to separate these diploid cells for DNA typing in forensic DNA analysis. The problem of separating similar cell types from individuals in a sexual assault can be solved by the differences in the sex chromosomes. A technique utilizing the sex chromosomes to separate the often seen and dreaded mixtures would be valuable to many forensic cases.

Fluorescence *in situ* hybridization (FISH) and laser microdissection (LMD) are both widely used techniques in research and in the medical field today. FISH allows the characterization of a wide range of molecular genetic events such as aneuploidy, gene deletion, and chromosome translocations and is achieved by labeling the DNA with a probe which emits a fluorescent signal. LMD is a rapid and precise method of isolating and physically removing specific cells from complex tissues which allows for such things as the assessment of cells in a particular disease. The combination of these two methods has yet to be applied to forensic casework. The application would enable the scientist to visually see the sex chromosomes and cut out the desired cells which would allow for the separation and analysis of samples from two individuals of different gender.

There are numerous technical aspects that had to be resolved before the actual experiments were performed. Adjustments to the FISH protocol, effects of FISH chemicals on PCR and DNA typing, and non-conventional slides for the LMD were all researched. After validation of the technical issues the mixture experiments proceeded. The experiments looked into the different ratios of male to female, loss of cells during FISH, contamination issues, and forensic-type samples. An experiment looking at the different ratio of male to female cells showed that the minor contributor in a sample can be identified in a mixture using FISH. It has also been determined that the majority of cells do stay on the slide during FISH which is important because some forensic cases have very little evidence. Use of FISH and LMD in simulated forensic samples and real-life cases has shown that the two techniques can be used successful and a DNA type can be obtained. The technique has the potential to benefit many forensic cases with

more profiles being entered in the database which could lead to more cases being solved.